

**Remarks/Arguments**

Claims 45-63, 65-74, 76-114 remain in the application. Claims 64, 75, and 88 - 111 have been cancelled and claims 45, 46, 47, 49, 54, 55, 56, 58, 60, 68, 69, 70, 71, 80, 81, 82, 87, 112, and 113 have been amended. Enabling support for the amendments can be found in the application as filed and in the previously pending claims, and therefore, no new matter or new issues are contained in the amendments. Reconsideration of the present application and allowance of the resulting Claims is respectfully requested in view of the amendments and following remarks.

**I. Rejection Under 35 U.S.C. §112 First Paragraph**

*A. Claims 45-51, 53-87, and 112-114*

Claims 45-51, 53-87, and 112-114 were rejected under 35 U.S.C. §112, first paragraph. In particular, the Office Action contends that the specification does not specifically define any of the gene sequences that fall within the genus nor does the specification define any structural features commonly possessed by members of the genus that distinguishes them from others. Applicants respectfully traverse this rejection.

Rubredoxin and  $\beta$ -amyloid are widely known by those skilled in the art, especially in regards to structure/form, source, and function. Applicants reiterate that the present invention is directed to a recombinant polynucleotide comprising a nucleotide sequence encoding a rubredoxin and  $\beta$ -amyloid fusion protein. The exact origin of the rubredoxin and  $\beta$ -amyloid are not critical to the nature of the invention. Applicants, in the previous response to Office Action, have supplied numerous publications detailing the source and function of rubredoxin and  $\beta$ -amyloid peptide.

In response, the Examiner cited to the *Eli Lilly* (43 USPQ2d 1398) case which does not apply to the present invention. In the *Eli Lilly* case, all mammalian forms of insulin were claimed by the Applicants but only the sequence of **one mammal was provided** at the time of the invention and no other sequences were known in the art. The present invention is not related to an area of art in which there is only one known protein or peptide sequence for rubredoxin or

$\beta$ -amyloid. One skilled in the art could construct numerous rubredoxin and  $\beta$ -amyloid fusion proteins based on the specification and the knowledge available in the art.

Further to the publications previously presented to the Examiner by the Applicants, which supports the numerous examples of known sequences of rubredoxin and  $\beta$ -amyloid peptides, Applicants enclose additional references which clearly support the fact that rubredoxin and  $\beta$ -amyloid are widely known in the art in regards to structure and function.

The Office Action suggests that the structure of rubredoxin is not supported in the specification; however, the structure of rubredoxin was widely known in the art at the time of filing. In Appendix A, Applicants enclose numerous sources to ascertain the structure for rubredoxin available at the time of filing the present application. The information supplied was obtained from www.pubmed.com. One skilled in the art could easily obtain 3-D structures of rubredoxin from *Pyrococcus furiosus*, *Desulfovibrio gigas*, *Clostridium pasteurianum*, *Desulfovibrio desulfuricans*, and *Desulfovibrio vulgaris* as seen in Appendix A. All of these 3-D structures were available well before the time of filing of this application, dating back to 1984 by K.D. Watenpaugh *et al.*

In Appendix B, Applicants enclose various sources to obtain the protein sequences for rubredoxin available at the time of filing the present application. Appendix B provides the sources and the sequences for rubredoxin which one of ordinary skilled in the art could obtain. The sources for the sequences of rubredoxin include *Azotobacter vinelandii*, by Menon *et al.* 1994; *Clostridium pasteurianum*, by Mathieu *et al.*, 1993; *Desulfovibrio vulgaris*, by Dolla A. *et al.* 1993; *Stenotrophomonas maltophilia*, by Lee N.R. *et al.* 1996; *Pyrococcus furiosus*, by Blake P.R., *et al.* 1993; *Heliobacillus mobilis*, by Lee W.Y. *et al.* 1995; *Desulfovibrio baarsii*, by Pianzola M.J., *et al.*, 1996; *Clostridium diolis*, by Gerard, P. Mar. 1997; *Clostridium acetobutylicum*, by Belouski, E. Mar. 1997; *Desulfovibrio gigas*, by Brushi, M., Sep. 1998; and *Desulfovibriode desulfuricans*, by Sieker, L.C. *et al.*, Oct. 1998. It is apparent from this non-exhaustive list that the sequence of rubredoxin and its various sources were widely known.

In Appendix C, Applicants enclose references from the database to ascertain the structures for  $\beta$ -amyloid peptide available at the time of filing the present application. Various structures of Beta-amyloid peptide were made available by J. Talaous *et al.* in 1994, M. Coles *et*

al. in April 1998, A.A. Watson et al. in April and June 1998 taken from studies of Alzheimer's disease, methionine oxidized, and NMR studies.

In Appendix D, Applicants enclose the various sources for the sequences of  $\beta$ -amyloid available at the time of filing the present application. The sources for the sequence of  $\beta$ -amyloid include *Mus musculus*, by Yamada, T. *et al.* 1993 and *Homo Sapiens*, by Zain, S.B. 1994. Other sequences of  $\beta$ -amyloid peptide are widely available in U.S. Patents Nos. 5,434,050 (filed on 1995), 5,441,870 (filed on 1995), 5,525,714 (filed on 1996), 5,552,426 (filed on 1996); and 5,605,811 (filed on 1997). All of these  $\beta$ -amyloid peptide sequences were published well before the filing of Applicants' application.

In Appendix E, Applicants resubmit the articles and publications supporting the widely known information relating to rubredoxin and  $\beta$ -amyloid available at the time of filing the present application, which are summarized in the table below.

<b>Crystallographic study of rubredoxin from the bacterium <i>Desulfovibrio desulfuricans</i> strain 27774.</b> Sieker LC, Jensen LH, Prickril BC, LeGall J <i>J Mol Biol</i> 1983 Nov 25 <b>171</b> :1 101-3
<b>Purification and properties of ferredoxin and rubredoxin from <i>Butyrivibacterium methylotrophicum</i>.</b> Saeki K, Jain MK, Shen GJ, Prince RC, Zeikus JG <i>J Bacteriol</i> 1989 Sep <b>171</b> :9 4736-41
<b>Expression of a synthetic gene coding for the amino acid sequence of <i>Clostridium pasteurianum</i> rubredoxin.</b> Eidsness MK, O Dell SE, Kurtz DM, Robson RL, Scott RA <i>Protein Eng</i> 1992 Jun <b>5</b> :4 367-71
<b>Synthesis and characterization of <i>Desulfovibrio gigas</i> rubredoxin and rubredoxin fragments.</b> Christensen HE, Hammerstad Pedersen JM, Holm A, Iversen G, Jensen MH, Ulstrup J <i>Eur J Biochem</i> 1994 Aug 15 <b>224</b> :1 97-101
<b>Isolation, characterization, and primary structure of rubredoxin from the photosynthetic bacterium, <i>Heliobacillus mobilis</i>.</b> Lee WY, Brune DC, LoBrutto R, Blankenship RE <i>Arch Biochem Biophys</i> 1995 Apr 1 <b>318</b> :1 80-8
<b>Recombinant two-iron rubredoxin of <i>Pseudomonas oleovorans</i>: overexpression, purification and characterization by optical, CD and <sup>113</sup>Cd NMR spectroscopies.</b> Lee HJ, Lian LY, Scrutton NS <i>Biochem J</i> 1997 Nov 15 <b>328</b> ( Pt 1): 131-6
<b><i>A Beta40 Is A Major Form Of Beta-Amyloid In Nonhuman Primates.</i></b> Gearing M, Tigges J, Mori H, Mirra SS <i>Neurobiol Aging</i> 1996 Nov-Dec <b>17</b> :903-8

<b><i>Amyloid Beta Protein In Rat Soleus Muscle In Chloroquine-Induced Myopathy Using End-Specific Antibodies For A Beta 40 And A Beta 42: Immunohistochemical Evidence For Amyloid Beta Protein.</i></b> Tsuzuki K, Fukatsu R, Takamaru Y, Yoshida T, Hayashi Y, Yamaguchi H, Fujii N, Takahata N Neurosci Lett 1995 Dec 202:77-80
<b><i>Homology Of The Amyloid Beta Protein Precursor In Monkey And Human Supports A Primate Model For Beta Amyloidosis In Alzheimer's Disease.</i></b> Podlisny MB, Tolan DR, Selkoe DJ Am J Pathol 1991 Jun 138:1423-35
<b><i>A Comprehensive Study Of The Spatiotemporal Pattern Of Beta-Amyloid Precursor Protein Mrna And Protein In The Rat Brain: Lack Of Modulation By Exogenously Applied Nerve Growth Factor.</i></b> Neve RL, Valletta JS, Li Y, Ventosa-Michelman M, Holtzman DM, Mobley WC Brain Res Mol Brain Res 1996 Jul 39:185-97
<b><i>Permeability And Residual Plasma Volume Of Human, Dutch Variant, And Rat Amyloid Beta-Protein 1-40 At The Blood-Brain Barrier.</i></b> Poduslo JF, Curran GL, Haggard JJ, Biere AL, Selkoe DJ Neurobiol Dis 1997 4:27-34
<b><i>Zinc-Induced Aggregation Of Human And Rat Beta-Amyloid Peptides In Vitro.</i></b> Esler WP, Stimson ER, Jennings JM, Ghilardi JR, Mantyh PW, Maggio JE J Neurochem 1996 Feb 66:723-32

As seen from these sources, Applicants should not be required to disclose each and every single sequences, structure, or function in the application as they are widely known by those skilled in the art.

As emphasized, Applicants' invention as currently claimed is related to the construct of the rubredoxin in combination with the  $\beta$ -amyloid peptide. Because both the genus and many examples of specific species within the genus are widely known in the art for each of rubredoxin and  $\beta$ -amyloid peptide, all that is required is that the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the presently claimed subject matter. *See In re Kaslow*, 707 F.2d 1366, 1375, 217 U.S.P.Q. 1089, 1096 (Fed. Cir. 1983).

Applicants reemphasize that the *Eli Lilly* case is fact specific and does not apply to the present invention, because rubredoxin and  $\beta$ -amyloid peptide are well known in the art in regards to form/structure, source, and function. Thus, as stated by the Federal Circuit "an inventor is not required to describe every detail of his invention. An applicant's disclosure obligation varies according to the art to which the invention pertains." *In re Hayes Microcomputer Products Inc. Patent Litigation*, 982 F.2d. 1527, 1534-35 (Fed. Cir. 1992). The prior art provides numerous



organisms which encode for rubredoxin from a wide variety of bacteria. At the time of the claimed invention, Applicants have stated that “rubredoxin from numerous different organism have been isolated, and the amino acid sequences of various rubredoxins and the genes encoding various rubredoxins have been published.” (Specification page 25, lines 27-29). In the present application, Applicants disclose the use of *D. vulagaris* as a source for a gene that encodes rubredoxin as a representative example, which is conventional in the art, and known to one of ordinary skill in the art. The selection of the source for encoding rubredoxin is sufficiently developed so as to put one of skill in the art in possession of the steps of the method and the elements of the composition. In other words, one skilled in the relevant art would understand what is intended by the claimed invention and how to carry it out. In light of the evidence submitted, one of ordinary skill in the art would understand the invention to be related to fusion protein comprising a rubredoxin fusion partner, fused directly or indirectly to a  $\beta$ -amyloid peptide, together with methods and materials for producing the fusion protein in a host cell and purifying the fusion protein, and not critically dependent upon the origin of the rubredoxin or  $\beta$ -amyloid in each case. The courts have pointed out that “[n]ot every last detail [of an invention need] be described [in a patent specification], else patent specifications would turn into production specifications, where there were never intended to be.” *In re Gay*, 309 F.2d 769, 774, 135 U.S.P.Q. 311, 316 (C.C.P.A. 1962).

Further, the claimed invention is not directed to a particular polynucleotide sequence; rather, the claims are directed to a construct describing a particular way of combining types of sequences (e.g., a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused  $\beta$ -amyloid peptide). The claims are therefore directed to a novel combination of known types of sequences, and are not drawn to a particular sequence. As such, Applicants are not required to disclose every possible sequence that can be used interchangeably in the claimed polynucleotide constructs. The novelty of the invention does not lie in the particular sequence used, but lies in the structure and combination of the construct. As least for the above mentioned remarks, Applicants respectfully request withdrawal of this rejection.

*B. Claim 52*

Claim 52 remains rejected under 35 U.S.C. §112, first paragraph, by the Examiner as “containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and or use the invention.” Applicants respectfully traverse this rejection.

The Examiner states that only a “schematic of the vector rRUBEX23 is described and only a portion of the sequence is disclosed...therefore, it would involve an undue experimentation to make this vector.” Applicants reiterate that claim 52 provides sufficient description that would not require undue experimentation to those of ordinary skilled in the art. Applicants reiterate that the specification clearly defines pRUBEX3, which comprises a histidine tag and a Factor Xa cleavage site of pRUBEX2 as well as containing “as part of the intervening spacer a portion of the multiple cloning region to facilitate cloning of the nucleotide sequence encoding the fused polypeptide into the vector”. pRUBEX2 and pRUBEX1 are in turn derived from the “coding sequence for *D. vulgaris* rubredoxin and the fused polypeptide are directly linked” (Specification, page 19, line 31 to page 20, line 9). As stated in the previous section, rubredoxin is well known in the art in regards to form, function, structure and source. Thus, one of ordinary skilled in the art could easily ascertain the coding sequence for coding sequence for *D. vulgaris* rubredoxin. Because the Examiner failed to consider the publications previously sent in support of these facts, Applicants submit and refer the Examiner to Appendix A, B, and D to represent that the coding sequence for rubredoxin which could be easily obtained from readily available source. Thus pRUBEX3 could be ascertained with the teachings of the specification because the source, sequence, and structure is widely known at the time of the present invention.

Applicants have also provided, in Figure 1, a schematic of the vector pRUBEX3, including the Multiple Cloning Region (MCR) and the nucleotide sequence of a portion of pRUBEX3 together with the amino acid sequence encoded. Not only is pRUBEX3 defined and exemplified in Figure 1, Applicants have also provided in Example 1, the method to yield pRUBEX3 (Specification, Example 1, page 25, line 23 to page 28, line 24; **with emphasis** on page 28, lines 23-24). Thus, the specification has disclosed in a manner that one skilled in the art would be able to practice the invention without an undue amount of experimentation. *See In re Colianni*, 561 F.2d at 224, 195 U.S.P.Q. at 153; *see also M.P.E.P* §2164.02.

The Examiner has also relied on 37 C.F.R. §1.802, as a basis for this rejection, which states:

(a) Where an invention is, or relies on, a biological material, the disclosure **may include** reference to a deposit of such biological material.

(b) Biological material need not be deposited unless access to such material is necessary for the satisfaction of the statutory requirements for patentability under 35 U.S.C. 112. **Biological material need not be deposited**, *inter alia*, if it is known and readily available to the public or **can be made or isolated without undue experimentation**.

(emphasis added; irrelevant sections omitted).

As stated above, Applicants are not required to submit the biological material if it can be made or isolated **without undue experimentation**. Applicants have articulated above that pRUBEX3 has been defined in the specification and exemplified in Figure 1 and Example 1. Because pRUBEX3 would not require an undue amount of experimentation to practice to those skilled in the art, Applicants are not required to deposit this biological material as it has been satisfied under the statutory requirements of 37 C.F.R. §1.802 and 35 U.S.C. §112. *See also Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d at 1210, 18 U.S.P.Q.2d at 1024 (inventor did not need to deposit cells used in the invention's best mode if one skilled in the art could reproduce without undue experimentation); *see also Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1385, 231 U.S.P.Q. at 94 (The mere fact that the best mode requires sophisticated competent people to engage in a labor-intensive and time-consuming process does not amount to a best mode violation in the absence of a deposit. The key to the analysis is whether the scientists must engage in *undue experimentation*).

Applicants have clearly enabled pRUBEX3 and have provided sufficient description and evidence to overcome the rejection. Thus, Applicants respectfully request withdrawal of this rejection.

**II. Rejection Under 35 U.S.C. §112, Second Paragraph**

*A. Claim 64*

The Office Action rejected claim 64 under 35 U.S.C. §112 which Applicants have cancelled and thus is no longer an issue. Applicants respectfully request withdrawal of this rejection.

*B. Claim 112*

The Office Action rejected claim 112, under 35 U.S.C. §112, second paragraph for being unclear. Applicants have amended claim 112 based on the Examiner's suggestion for clarity and thus respectfully request withdrawal of this rejection.

**III. Rejection Under 35 U.S.C. §103**

The Examiner has rejected claims 45-51, 53-63, 65-87, and 112-114 under 35 U.S.C. §103(a) as unpatentable over Ueno *et al.* and Dobeli *et al.* Applicants respectfully traverse this rejection.

Applicants have amended the claims to further highlight the significance of the solubility of the rubredoxin and the preferred protein of interest  $\beta$ -amyloid fusion protein in a recombinant expression system. Applicants emphasize that the Federal Circuit has held that evidence of a teaching, suggestion, or motivation to combine may flow from references themselves or from the knowledge of one of ordinary skilled in the art, but may **not** flow from the Applicants' disclosure. See *Pro-Mold Tool Co. v. Great Lakes Plastics, Inc.*, 75 F.3d 1568, 1573 (Fed. Cir. 1996), *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). Importantly, this evidence of a teaching, suggestion, or motivation, to combine must be "clear and particular". *In re Dembiczak*, 175 F.3d 994, 999 (Fed. Cir. 1999).

In the present case, the Examiner has not pointed to any actual evidence in the cited references that would have led one of ordinary skill in the art to combine the teachings in a manner suggested by the Examiner. As stated by the Federal Circuit, this evidence must be clear and particular. Broad conclusory statements regarding the teachings of multiple references, standing alone, are not "evidence". *Id.* In the outstanding Office Action, the Examiner has used the teachings of the present invention to show that it would have been obvious for Ueno *et al.* to

combine rubredoxin with the  $\beta$ -amyloid taught by Dobeli *et al.* Neither references, alone or in combination, suggests a soluble rubredoxin-B-amyloid combination as presently claimed.

Applicants emphasize the unexpected benefits of the present invention with respect to the particular rubredoxin and  $\beta$ -amyloid fusion construct now claimed. The Dobeli *et al.* 13.8 kD fusion with the amyloid is not soluble and goes into the inclusion bodies. Further, nowhere in the reference does it teach or suggest an iron containing nucleotide having red color for easy tracking. Not only is there no suggestion or motivation, the Dobeli *et al.* reference teaches away from the presently claimed invention. The Dobeli reference teaches a chemical cleavage of beta-amyloid from fusion which causes side reactions. The Ueno *et al.* reference does not teach or suggest the use of any  $\beta$ -amyloid. Further the Ueno disclosure does not teach rubredoxin constituent that is soluble, nor does it teach a rubredoxin constituent that goes into the inclusion bodies. Thus, even if all the references were to be combined, there would be no teaching or suggestion of the claimed invention. Prior to the present application, no one had described that rubredoxin was an advantageous fusion partner for creating soluble  $\beta$ -amyloid expression products that are also colorimetric.

Applicants highlight the Kohli *et al* reference<sup>1</sup> (previously provided) only to show that the presently claimed invention is novel and not obvious. Five years after the filing of the present application, Kohli *et al* published their findings of surprising utility of rubredoxin expression system as claimed by the present invention. Based, on the foregoing remarks, Applicants respectfully request withdrawal of this rejection.

#### **IV. Conclusion**

In summary, Applicants have particularly pointed out and distinctly claimed the subject matter which Applicants regard as the invention, and the subject matter of the present invention is described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Furthermore, the subject matter of the present invention is neither disclosed nor rendered obvious by any of the cited documents or by any combination of the documents if the

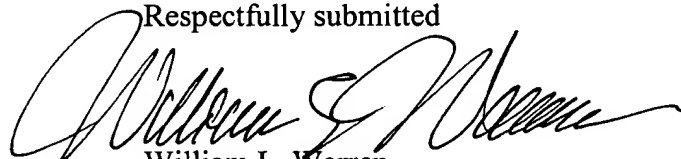
---

<sup>1</sup> Kohli, Bernhard *et al.*, *Protein Expression & Purification* 28 (2003) 362-367.

skilled person would have combined them at all. Therefore, Applicants respectfully request withdrawal of the present rejections and allowance of the claims.

The Examiner is encouraged to call the undersigned attorney at 404-853-8081 if doing so will facilitate prosecution of the application. No fees are believed to be due at this time. However, the Commissioner is hereby authorized to charge any additional fees due or credit any overpayment to Deposit Account 19-5029.

In view of the present amendment and response to the Office Action mailed August 24, 2004, Applicant respectfully requests a timely Notice of Allowance be issued in this case.

Respectfully submitted  
  
William L. Warren  
Reg. No. 36,714

SUTHERLAND ASBILL & BRENNAN LLP  
999 Peachtree Street, NE  
Atlanta, Georgia 30309-3996  
Telephone: (404) 853-8000  
Facsimile: (404) 853-8806





PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Rubredoxin (Oxidized).

**Deposition:** M.W.Day, B.T.Hsu, L.Joshua-Tor, J.B.Park, Z.H.Zhou, M.W.W.Adams & D.C.Rees, 18-May-92

**Taxonomy:** Pyrococcus furiosus

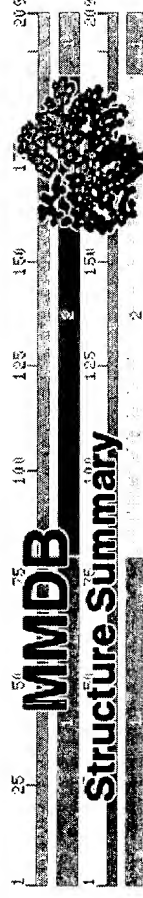
**Reference:** PubMed **MMDB:** 438 **PDB:** 1CAA

[View 3D Structure](#) of [Best Model](#) with [Cn3D](#) [Display](#) [NEW](#) [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", **Nucleic Acids Res.** 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Rubredoxin (Zn-Substituted) (Nmr, 40 Structures).

**Deposition:** P.R.Blake, J.B.Park, Z.H.Zhou, D.R.Hare, M.W.W.Adams & M.F.Summers, 10-Jul-92

**Taxonomy:** Pyrococcus furiosus

**Reference:** PubMed **MMDB:** 2499 **PDB:** 1ZRP

of

with

[Get Cn3D 4.1I](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLN](#) | [NIH](#)





PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Rubredoxin (Reduced).

**Deposition:** M.W.Day, B.T.Hsu, L.Joshua-Tor, J.B.Park, Z.H.Zhou, M.W.W.Adams & D.C.Rees, 18-May-92

**Taxonomy:** Pyrococcus furiosus

**Reference:** PubMed **MMDB:** 439 **PDB:** 1CAD

of  with



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLN](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

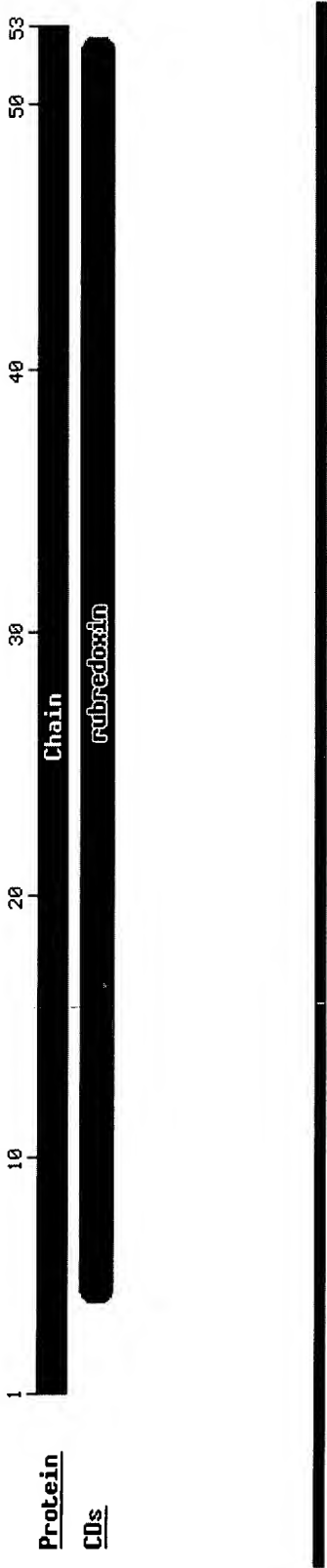
Description: Rubredoxin.

Deposition: M.Frey, L.C.Sieker & F.Payan, 17-Mar-88

Taxonomy: Desulfovibrio gigas

Reference: PubMed MMDB: 2057 PDB: 1RDG

of  with



Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Rubredoxin (Oxidized, Fe(III)) (Unconstrained Model).

**Deposition:** K.D.Watenpugh, L.C.Sieker & L.H.Jensen, 15-Oct-84

**Taxonomy:** *Clostridium pasteurianum*

**Reference:** [PubMed](#) **MMDB:** [3211](#) **PDB:** [4RXN](#)

of  with    [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

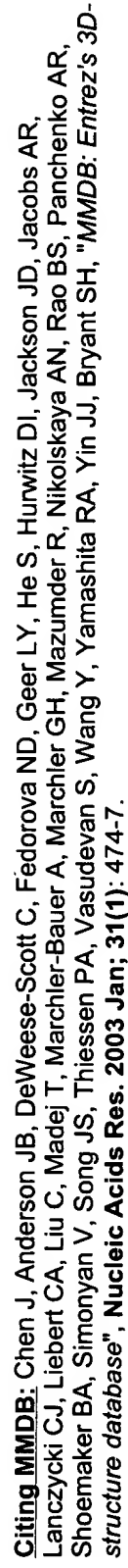
[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



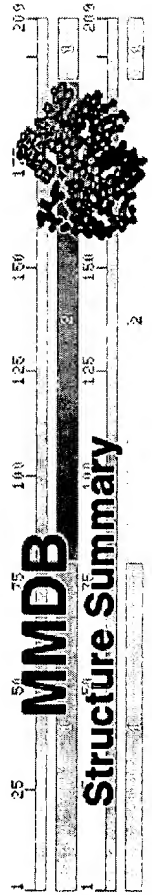

Cn3d

**Reference:** PubMed **MMDB:** 3292 **PDB:** 5RXN

## Get Cn3D 4.1!



11/15/2004



PubMed

BLAST

Structure

Taxonomy

OMIM

Help?

Cn3d

**Description:** Rubredoxin.

**Deposition:** R.E.Stenkamp, L.C.Sieker & L.H.Jensen, 16-Jan-90

**Taxonomy:** [Desulfovibrio desulfuricans](#)

**Reference:** [PubMed](#) **MMDB:** [3344](#) **PDB:** [6RXN](#)

View 3D Structure

of

Best Model

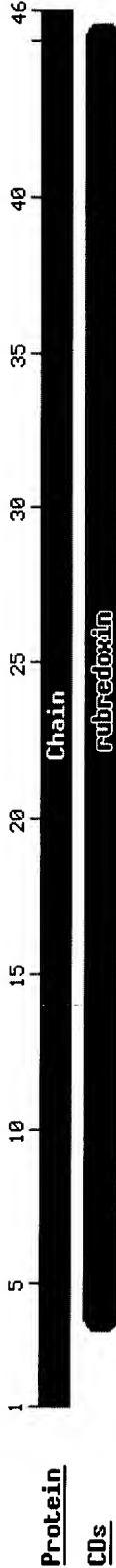
with

Cn3D

Display

NEW

Get Cn3D 4.1!



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", **Nucleic Acids Res.** 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

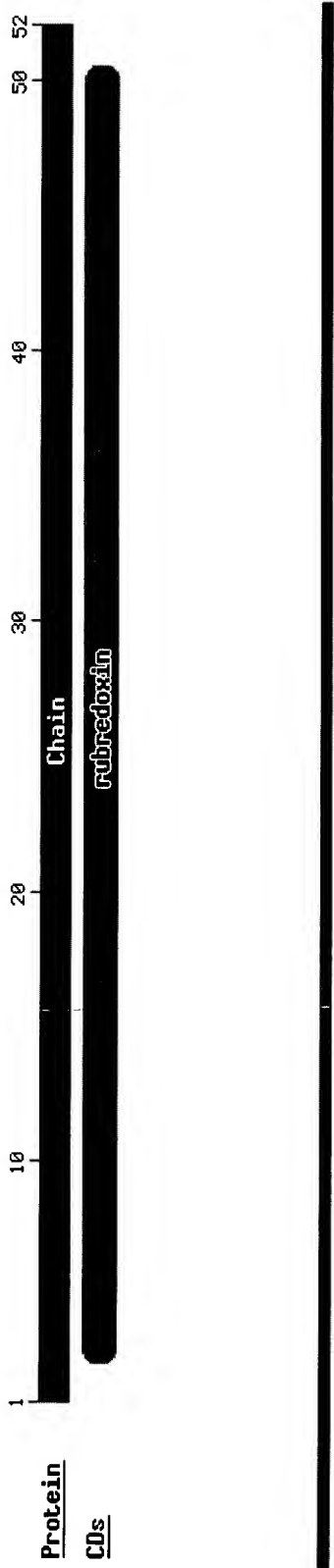
Description: Rubredoxin.

Deposition: E.T.Adman, L.C.Sieker & L.H.Jensen, 11-May-90

Taxonomy: Desulfovibrio vulgaris

Reference: PubMed **MMDB: 3382** **PDB: 7RXN**

of  with    [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", **Nucleic Acids Res.** 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

Description: Rubredoxin.

Deposition: Z.Dauter, L.Sieker & K.Wilson, 26-Aug-91

Taxonomy: Desulfovibrio vulgaris

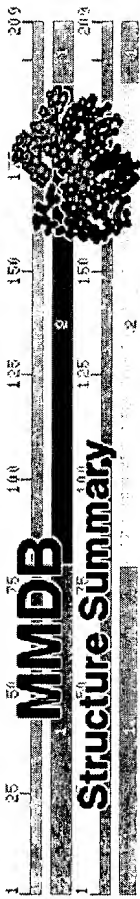
Reference: PubMed **MMDB: 3418** **PDB: 8RXN**

of  with    [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", **Nucleic Acids Res.** 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Rubredoxin (Oxidized, Fe(lII)) At 1.1 Angstroms Resolution.

**Deposition:** Z.Dauter, K.S.Wilson, L.C.Sieker, J.M.Moulis & J.Meyer, 13-Dec-95

**Taxonomy:** Clostridium pasteurianum

**Reference:** PubMed **MMDB:** 4287 **PDB:** 1IRO

of  with    [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)







PubMed   BLAST   Structure   Taxonomy   OMIM   Help?   Cn3d

**Description:** Rubredoxin (Zn-Substituted) At 1.2 Angstroms Resolution.

**Deposition:** Z.Dauter, K.S.Wilson, L.C.Sieker, J.M.Moulis & J.Meyer, 13-Dec-95

**Taxonomy:** *Clostridium pasteurianum*

**Reference:** [PubMed](#)   **MMDB:** [4288](#)   **PDB:** [1IRN](#)

of
with



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)





PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Rubredoxin (Wild Type) From *Pyrococcus Furiosus*.  
**Deposition:** R.Bau, D.C.Rees, D.M.Kurtz, R.A.Scott, H.Huang, M.W.W.Adams & M.K.Eidsness, 24-Aug-98  
**Taxonomy:** *Pyrococcus furiosus*  
**Reference:** [PubMed](#) **MMDB:** [8262](#) **PDB:** [1BRF](#)

of  with    [Get Cn3D 4.1!](#)

**Protein**  
**CDs**

1 10 20 30 40 50 53

Chain A  
rubredoxin

**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)





PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Clostridium Pasteurianum Rubredoxin C42s Mutant.  
**Deposition:** M.Maher, J.M.Guss, M.Wilce & A.G.Wedd, 20-May-98  
**Taxonomy:** Clostridium pasteurianum  
**Reference:** [PubMed](#) **MMDB:** [8416](#) **PDB:** [1BE7](#)

[View 3D Structure](#) of [Best Model](#) with [Cn3D](#) [Display](#) [NEW](#) [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



MMDB

Structure Summary

1 25 50 75 100 125 150 175 200



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

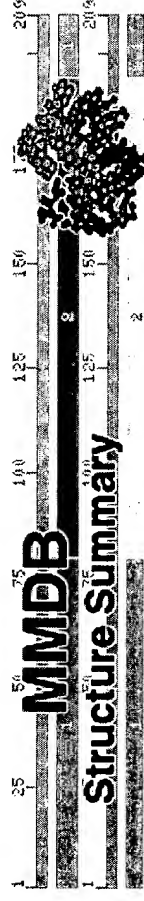
**Description:** Rubredoxin From Desulfovibrio Vulgaris Refined Anisotropically At 0.92 Angstroms Resolution.  
**Deposition:** Z.Dauter, S.Butterworth, L.C.Sieker, G.Sheldrick & K.S.Wilson, 21-Dec-97  
**Taxonomy:** Desulfovibrio vulgaris  
**Reference:** PubMed **MMDB:** 9637 **PDB:** 1RB9

View 3D Structure of Best Model with Cn3D Display    Get Cn3D 4.1!



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

Disclaimer | Write to the Help Desk  
NCBI | NLM | NIH



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Clostridium Pasteurianum Rubredoxin G10a Mutant.

**Deposition:** M.J.Maher, J.M.Guss, M.C.J.Wilce & A.G.Wedd, 26-Nov-98

**Taxonomy:** Clostridium pasteurianum

**Reference:** PubMed **MMDB:** 10186 **PDB:** 1B13

of  with



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Clostridium Pasteurianum Rubredoxin G43a Mutant.

**Deposition:** M.J.Maher, J.M.Guss, M.C.J.Wilce & A.G.Wedd, 27-Nov-98

**Taxonomy:** Clostridium pasteurianum

**Reference:** PubMed **MMDB:** 10187 **PDB:** 1B2J

View 3D Structure

of

Best Model

with

Cn3D

Display

NEW

Get Cn3D 4.1!



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

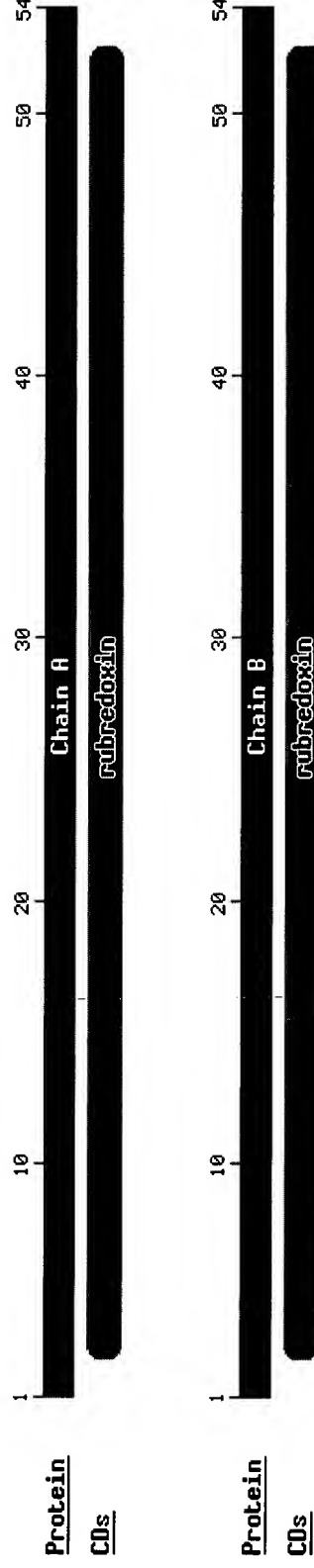
**Description:** Clostridium Pasteurianum Rubredoxin G10vg43a Mutant.

**Deposition:** M.J.Maher, J.M.Guss, M.C.J.Wilce & A.G.Wedd, 30-Nov-98

**Taxonomy:** Clostridium pasteurianum

**Reference:** PubMed [MMDB: 10188](#) [PDB: 1B2O](#)

[View 3D Structure](#) of [Best Model](#) with [Cn3D](#) [Display](#) [NEW!](#) [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)





PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Solution Structure Of Reduced Clostridium Pasteurianum Rubredoxin, Nmr, 20 Structures.

**Deposition:** I.Bertini, D.M.Kurtz Junior, M.K.Eidsness, G.Liu, C.Luchinat, A.Rosato & R.A.Scott, 23-May-98

**Taxonomy:** [Clostridium pasteurianum](#)

**Reference:** [PubMed](#) **MMDB:** [10206](#) **PDB:** [1BFY](#)

View 3D Structure

 of 

Best Model

 with 

Cn3D

Display

NEW!

Get Cn3D 4.1!



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Rubredoxin From Desulfovibrio Vulgaris Miyazaki F, Trigonal Crystal Form.

**Deposition:** Y.Higuchi & N.Yasuoka, 30-Sep-98

**Taxonomy:** Desulfovibrio vulgaris

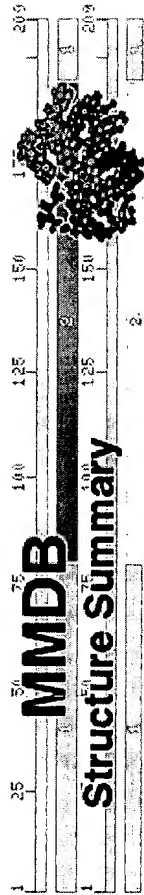
**Reference:** PubMed **MMDB:** 10323 **PDB:** 1RDV

of  with    [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lancycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Rubredoxin From Desulfovibrio Vulgaris Miyazaki F, Monoclinic Crystal Form.

**Deposition:** Y.Higuchi & N.Yasuoka, 7-Oct-98

**Taxonomy:** Desulfovibrio vulgaris

**Reference:** PubMed [MMDB: 10357](#) PDB: [2RDV](#)

of  with    [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", **Nucleic Acids Res.** 2003 Jan; **31**(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)





PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Rubredoxin (Methionine Mutant) From *Pyrococcus Furiosus*.  
**Deposition:** R.Bau, D.C.Rees, D.M.Kurtz, R.A.Scott, H.Huang, M.W.W.Adams & M.K.Eidsness, 22-Aug-98  
**Taxonomy:** *Pyrococcus furiosus*  
**Reference:** PubMed **MMDB:** 11956 **PDB:** 1BQ8

[View 3D Structure](#) of [Best Model](#) with [Cn3D](#) [Display](#) [NEW](#) [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)





PubMed

BLAST

Structure

Taxonomy

OMIM

Help?

Cn3d

**Description:** Rubredoxin (Formyl Methionine Mutant) From *Pyrococcus Furiosus*.  
**Deposition:** R.Bau, D.C.Rees, D.M.Kurtz, R.A.Scott, H.Huang, M.W.W.Adams & M.K.Eidsness, 22-Aug-98  
**Taxonomy:** *Pyrococcus furiosus*  
**Reference:** [PubMed](#) **MMDB:** [11957](#) **PDB:** [1BQ9](#)

View 3D Structure

 of 

Best Model

 with 

Cn3D

Display


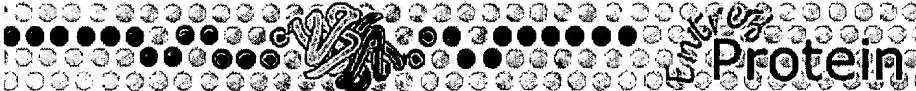
NEW

Get Cn3D 4.1!



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

☐ 1: [AAA19505](#). Reports rubredoxin...[gi:398004] [BLink, Domains, Links](#)

LOCUS            AAA19505                            72 aa                    linear    BCT 01-JUL-1994  
 DEFINITION     rubredoxin.  
 ACCESSION      AAA19505  
 VERSION        AAA19505.1    GI:398004  
 DBSOURCE       locus AVIHOXHYP accession [L23970.1](#)  
 KEYWORDS       .  
 SOURCE          Azotobacter vinelandii  
     ORGANISM    *Azotobacter vinelandii*  
                  Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;  
                  Pseudomonadaceae; Azotobacter.  
 REFERENCE      1 (residues 1 to 72)  
     AUTHORS     Menon,A.L., Stults,L.W., Robson,R.L. and Mortenson,L.E.  
     TITLE        Cloning, sequencing and characterization of the  
                  [NiFe]hydrogenase-encoding structural genes (hoxK and hoxG) from  
                  Azotobacter vinelandii  
     JOURNAL      Gene 96 (1), 67-74 (1990)  
     MEDLINE      [91092503](#)  
     PUBMED       [2265761](#)  
 REFERENCE      2 (residues 1 to 72)  
     AUTHORS     Chen,J.C. and Mortenson,L.E.  
     TITLE        Two open reading frames (ORFs) identified near the hydrogenase  
                  structural genes in Azotobacter vinelandii, the first ORF may  
                  encode for a polypeptide similar to rubredoxins  
     JOURNAL      Biochim. Biophys. Acta 1131 (1), 122-124 (1992)  
     MEDLINE      [92256484](#)  
     PUBMED       [1581355](#)  
 REFERENCE      3 (residues 1 to 72)  
     AUTHORS     Chen,J.C. and Mortenson,L.E.  
     TITLE        Identification of six open reading frames from a region of the  
                  Azotobacter vinelandii genome likely involved in dihydrogen  
                  metabolism  
     JOURNAL      Biochim. Biophys. Acta 1131 (2), 199-202 (1992)  
     MEDLINE      [92305060](#)  
     PUBMED       [1610901](#)  
 REFERENCE      4 (residues 1 to 72)  
     AUTHORS     Menon,A.L., Mortenson,L.E. and Robson,R.L.  
     TITLE        Nucleotide sequences and genetic analysis of hydrogen oxidation  
                  (hox) genes in Azotobacter vinelandii  
     JOURNAL      J. Bacteriol. 174 (14), 4549-4557 (1992)  
     MEDLINE      [92325046](#)  
     PUBMED       [1624446](#)  
 REFERENCE      5 (residues 1 to 72)  
     AUTHORS     Garg,R.P., Menon,A.L., Jacobs,K., Robson,R.M. and Robson,R.L.  
     TITLE        The hypE gene completes the gene cluster for H<sub>2</sub>-oxidation in  
                  Azotobacter vinelandii  
     JOURNAL      J. Mol. Biol. 236 (1), 390-396 (1994)  
     MEDLINE      [94149737](#)  
     PUBMED       [7906310](#)

COMMENT      Method: conceptual translation.

FEATURES      Location/Qualifiers

source	1..72
	/organism="Azotobacter vinelandii"
	/db_xref="taxon:354"
Protein	1..72
	/product="rubredoxin"
Region	22..70
	/region_name="Rubredoxin"
	/note="rubredoxin"
	/db_xref="CDD:24175"
CDS	1..72
	/gene="hoxR"
	/coded_by="L23970.1:6066..6284"
	/citation=[2]
	/citation=[4]
	/transl_table=11

ORIGIN

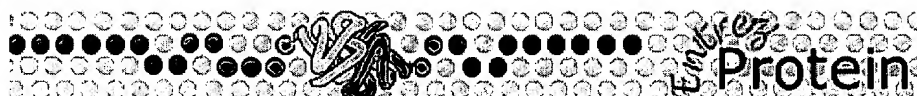
1	msarfe	gysyl	gd	trl	ladda	vle	ckic	wqr	yd	pa	eg	dp	vw	qip	pg	tp	faa	lp	ah	w	r	c	p	r	c
61	dg	d	r	e	q	f	m	v	v	dg															

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10





Entrez PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: AAA23279. Reports rubredoxin...[gi:144909]BLink, Domains,  
Links

LOCUS AAA23279 54 aa linear BCT 26-APR-1993

DEFINITION rubredoxin.

ACCESSION AAA23279

VERSION AAA23279.1 GI:144909

DBSOURCE locus CLORUB accession M60116.1

KEYWORDS .

SOURCE Clostridium pasteurianum

ORGANISM Clostridium pasteurianum

Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
Clostridium.

REFERENCE 1 (residues 1 to 54)

AUTHORS Mathieu, I., Meyer, J. and Moulis, J.M.

TITLE Cloning, sequencing and expression in Escherichia coli of the  
rubredoxin gene from Clostridium pasteurianum

JOURNAL Biochem. J. 285 (Pt 1), 255-262 (1992)

MEDLINE 92344580

PUBMED 1637309

COMMENT Method: conceptual translation.

FEATURES Location/Qualifiers

source

1..54

/organism="Clostridium pasteurianum"

/db\_xref="taxon:1501"

Protein

1..54

/product="rubredoxin"

Region

3..52

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

CDS

1..54

/coded\_by="M60116.1:2157..2321"

/note="sequence of product differs from reported protein  
sequence (accession number in NBRF-PIR: RUCLEP) in three  
positions: D14->N, D22->N, E48->Q"

/evidence=experimental

/transl\_table=11


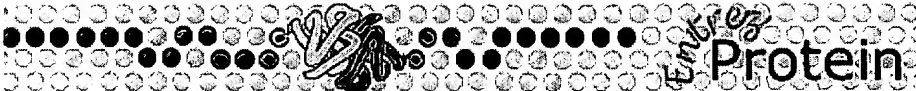
ORIGIN

1 mkkylctvcg yiynpedgdp dngvnpgtdf kdipddwvcp lcgvgkdqfe evee

//

[Disclaimer](#) | [Write to the Help Desk](#)[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

Limits     Preview/Index    History    Clipboard    Details

☐ 1: [AAA23380](#). Reports rubredoxin oxidor...[gi:145115]

LOCUS AAA23380 126 aa linear BCT 26-APR-1993

DEFINITION rubredoxin oxidoreductase.

ACCESSION AAA23380

VERSION AAA23380.1 GI:145115

DBSOURCE locus DVURBORUB accession [M81168.1](#)

KEYWORDS .

SOURCE Desulfovibrio vulgaris

ORGANISM [Desulfovibrio vulgaris](#)

Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1 (residues 1 to 126)

AUTHORS Dolla,A., Fu,R., Brumlik,M.J. and Voordouw,G.

TITLE Nucleotide sequence of dcrA, a Desulfovibrio vulgaris Hildenborough chemoreceptor gene, and its expression in Escherichia coli

J. Bacteriol. 174 (6), 1726-1733 (1992)

MEDLINE [92193255](#)

PUBMED [1548224](#)

COMMENT Method: conceptual translation.

FEATURES Location/Qualifiers

source

1..126

/organism="Desulfovibrio vulgaris"

/strain="Hildenborough"

/db\_xref="taxon:881"

/tissue\_lib="NCIMB 8303"

Protein

1..126

/product="rubredoxin oxidoreductase"

Region

2..37

/region\_name="Desulfoferrodoxin, N-terminal domain"

/note="Desulfoferrod\_N"

/db\_xref="CDD:26510"

Region

41..125

/region\_name="Desulfoferrodoxin"

/note="Desulfoferrodox"

/db\_xref="CDD:25838"

CDS

1..126

/gene="rbo"

/coded\_by="M81168.1:2529..2909"

/transl\_table=11

ORIGIN

1 mpnqyeiyc ihcg nivevl hagg gdlvcc gepmklmkeg tsdgakekhv pviektangy  
61 kvtvgsvahp meekhwiewi elvadgvsyk kflkpgdape aefcikadkv vareycnlhg  
121 hwkaea

//

Nov 8 2004 13:44:10

**THIS PAGE BLANK (USPTO)**



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Bio

Search Protein  for 

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default Show: 20 

Send to

File 

Get Subsequence


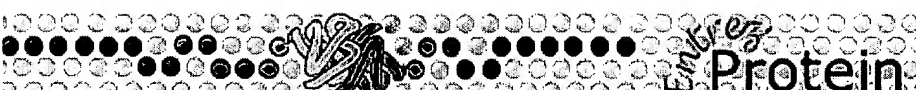
Feat

☐ 1: AAA23381. Reports rubredoxin...[gi:145116]BLink, Domains,  
Links

LOCUS AAA23381 52 aa linear BCT 26-APR-1993  
 DEFINITION rubredoxin.  
 ACCESSION AAA23381  
 VERSION AAA23381.1 GI:145116  
 DBSOURCE locus DVURBORUB accession M81168.1  
 KEYWORDS .  
 SOURCE Desulfovibrio vulgaris  
 ORGANISM Desulfovibrio vulgaris  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.  
 REFERENCE 1 (residues 1 to 52)  
 AUTHORS Dolla,A., Fu,R., Brumlik,M.J. and Voordouw,G.  
 TITLE Nucleotide sequence of dcrA, a Desulfovibrio vulgaris Hildenborough  
 chemoreceptor gene, and its expression in Escherichia coli  
 JOURNAL J. Bacteriol. 174 (6), 1726-1733 (1992)  
 MEDLINE 92193255  
 PUBMED 1548224  
 COMMENT Method: conceptual translation.  
 FEATURES Location/Qualifiers  
 source 1..52  
 /organism="Desulfovibrio vulgaris"  
 /strain="Hildenborough"  
 /db\_xref="taxon:881"  
 /tissue\_lib="NCIMB 8303"  
 Protein 1..52  
 /product="rubredoxin"  
 /function="catalyzes the reaction H2 = 2H+ + 2e"  
 Region 3..50  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 CDS 1..52  
 /gene="rub"  
 /coded\_by="M81168.1:2926..3084"  
 /note="Rubredoxin is a monomer"  
 /transl\_table=11  
 ORIGIN  
 1 mkkyvctvcg yeydpaegdp dngvkpgtsf ddlpadwvcp vcgapksefe aa  
 //

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

Limits   Preview/Index   History   Clipboard   Details

Show:

☐ 1: [AAA64797](#). Reports rubredoxin oxidor...[gi:758676] BLink, Domains, Links

LOCUS      AAA64797                      126 aa                      linear      BCT 05-APR-1995  
 DEFINITION rubredoxin oxidoreductase.  
 ACCESSION   AAA64797  
 VERSION      AAA64797.1    GI:758676  
 DBSOURCE     locus DVURUBRBO accession [M28848.1](#)  
 KEYWORDS      .  
 SOURCE        Desulfovibrio vulgaris  
                 ORGANISM   [Desulfovibrio vulgaris](#)  
                                 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
                                 Desulfovibrionaceae; Desulfovibrio.  
 REFERENCE    1 (residues 1 to 126)  
                 AUTHORS   Brumlik,M.J. and Voordouw,G.  
                 TITLE        Analysis of the transcriptional unit encoding the genes for  
                                 rubredoxin (rub) and a putative rubredoxin oxidoreductase (rbo) in  
                                 Desulfovibrio vulgaris Hildenborough  
                 JOURNAL     J. Bacteriol. 171 (9), 4996-5004 (1989)  
                 MEDLINE     [89359139](#)  
                 PUBMED      [2549009](#)  
 COMMENT      Method: conceptual translation.  
 FEATURES      Location/Qualifiers  
                 source            1..126  
                                         /organism="Desulfovibrio vulgaris"  
                                         /strain="Hildenborough"  
                                         /db\_xref="taxon:881"  
                 Protein            1..126  
                                         /product="rubredoxin oxidoreductase"  
                 Region            2..37  
                                         /region\_name="Desulfoferrodoxin, N-terminal domain"  
                                         /note="Desulfoferrodox\_N"  
                                         /db\_xref="CDD:26510"  
                 Region            41..125  
                                         /region\_name="Desulfoferrodoxin"  
                                         /note="Desulfoferrodox"  
                                         /db\_xref="CDD:25838"  
                 CDS                1..126  
                                         /gene="rbo"  
                                         /coded\_by="M28848.1:199..579"  
                                         /transl\_table=11

ORIGIN  
       1 mpnqyeiyc ihcgnivevl hagggdvcc gepmklmkeg tsdgakekhv pviectangy  
       61 kvvtgsvahp meekhwiewi elvadgvsyk kflkpgdape aefcikadkv vareycnlhg  
       121 hwkaea  
 //

Nov 8 2004 13:44:10

**THIS PAGE BLANK (USPTO)**



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

1: AAA64798. Reports rubredoxin...[gi:758677]

BLink, Domains,  
Links

LOCUS AAA64798 52 aa linear BCT 05-APR-1995  
 DEFINITION rubredoxin.  
 ACCESSION AAA64798  
 VERSION AAA64798.1 GI:758677  
 DBSOURCE locus DVURUBRBO accession M28848.1  
 KEYWORDS .  
 SOURCE Desulfovibrio vulgaris  
 ORGANISM Desulfovibrio vulgaris  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.  
 REFERENCE 1 (residues 1 to 52)  
 AUTHORS Brumlik,M.J. and Voordouw,G.  
 TITLE Analysis of the transcriptional unit encoding the genes for  
 rubredoxin (rub) and a putative rubredoxin oxidoreductase (rbo) in  
 Desulfovibrio vulgaris Hildenborough  
 JOURNAL J. Bacteriol. 171 (9), 4996-5004 (1989)  
 MEDLINE 89359139  
 PUBMED 2549009  
 COMMENT Method: conceptual translation.  
 FEATURES Location/Qualifiers  
 source 1..52  
 /organism="Desulfovibrio vulgaris"  
 /strain="Hildenborough"  
 /db\_xref="taxon:881"  
 Protein 1..52  
 /product="rubredoxin"  
 Region 3..50  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 CDS 1..52  
 /gene="rub"  
 /coded\_by="M28848.1:596..754"  
 /transl\_table=11  
 ORIGIN  
 1 mkkyvctvcg yeydpaegdp dngvkpgtsf ddlpadwvcp vcgapksefe aa  
 //

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Bio

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

1: AAA97867. Reports rubredoxin reduct...[gi:1276869]

BLink, Links

LOCUS AAA97867 264 aa linear BCT 25-APR-1996

DEFINITION rubredoxin reductase.

ACCESSION AAA97867

VERSION AAA97867.1 GI:1276869

DBSOURCE locus SMU40234 accession U40234.1

KEYWORDS

SOURCE Stenotrophomonas maltophilia

ORGANISM Stenotrophomonas maltophilia

Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales;  
Xanthomonadaceae; Stenotrophomonas.

REFERENCE 1 (residues 1 to 264)

AUTHORS Lee,N.R., Hwang,M.O., Jung,G.H., Kim,Y.S. and Min,K.H.

TITLE Physical structure and expression of alkBA encoding alkane  
hydroxylase and rubredoxin reductase from Pseudomonas maltophilia

JOURNAL Biochem. Biophys. Res. Commun. 218 (1), 17-21 (1996)

MEDLINE 96136269

PUBMED 8573125

REFERENCE 2 (residues 1 to 264)

AUTHORS Min,K.-H.

TITLE Direct Submission

JOURNAL Submitted (07-NOV-1995) Kyung-Hee Min, Sookmyung Women's  
University, Department of Biology, Chungpa-Dong 2-Ka, Yongsan-Ku,  
Seoul 140-742, Korea

COMMENT Method: conceptual translation.

FEATURES Location/Qualifiers

source

1..264

/organism="Stenotrophomonas maltophilia"

/strain="N246"

/db\_xref="taxon:40324"

/clone="pPOC122"

/clone\_lib="library of K.-H. Min"

Protein

1..264

/product="rubredoxin reductase"

CDS

1..264

/gene="alkA"

/coded\_by="U40234.1:1..>794"

/transl\_table=11

ORIGIN

```

1 mveyycplcv iydtnkgfps fgmhlfswn d teidescpdl mvrlldfkkl spfkftqlgm
61 glasenlisd ititglansa lalkyplfnf nkieicankt gvllslrlvv eksinklmli
121 ksasiqlpde levfdysvs eptptflnpi kwhlpdfsvk ldfildfsgk lvlsqdtphl
181 sfvilnsqta tvcapftiek lmsatvadlf ktdiprvagg hiacewrcik mgmydeylty
241 eafftpgtis idipqdqccy ianv

```

//

[Disclaimer](#) | [Write to the Help Desk](#)

[NCBI](#) | [NLM](#) | [NIH](#)



Nov 8 2004 13:44:10

**THIS PAGE BLANK (USPTO)**



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein  for 

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 1606208A. Reports rubredoxin...[gi:226794]BLink, Domains,  
Links

LOCUS 1606208A 52 aa linear BCT 21-OCT-1996  
 DEFINITION rubredoxin.  
 ACCESSION 1606208A  
 VERSION 1606208A GI:226794  
 DBSOURCE prf: locus 1606208A;

state: embryo;

taxonomy: Prokaryota.

KEYWORDS Rubredoxin; Desulfovibrio vulgaris; Purifn; LC; CPase Y/A Digest;  
 Seq Determination; 52AAs; For-Met at N Term; Seq Comparison; Redox  
 Potential of 5mV; Seq Comparison with Cytochrome c.

SOURCE Desulfovibrio vulgaris

ORGANISM Desulfovibrio vulgaris

Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1 (residues 1 to 52)

AUTHORS Shimizu,F., Ogata,M., Yagi,T., Wakabayashi,S. and Matsubara,H.

TITLE Amino acid sequence and function of rubredoxin from Desulfovibrio  
vulgaris Miyazaki

JOURNAL Biochimie 71(11/12), 1171-1177 (1989)

COMMENT N.For:EC=4.1.1.39.

FEATURES Location/Qualifiers

source

1..52

/organism="Desulfovibrio vulgaris"

/db\_xref="taxon:881"

Region

3..51

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

ORIGIN

1 mkkyvctvcg yeydpaegdp dngvkgptaf edvpadwvcp icgapksefe pa

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 1924243A. Reports rubredoxin...[gi:738104]BLink, Domains,  
Links

LOCUS 1924243A 75 aa linear BCT 21-NOV-1996  
 DEFINITION rubredoxin.  
 ACCESSION 1924243A  
 VERSION 1924243A GI:738104  
 DBSOURCE prf: locus 1924243A;

state: embryo;  
 taxonomy: Prokaryota.

KEYWORDS Rubredoxin; Clostridium pasteurianum; Transcription Mapping; Seq  
 Determination; ORF 2; Seq Comparison.

SOURCE Clostridium pasteurianum

ORGANISM Clostridium pasteurianum

Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 75)

AUTHORS Mathieu, I. and Meyer, J.

TITLE Transcript mapping of the rubredoxin gene from Clostridium  
 pasteurianum

JOURNAL FEMS Microbiol. Lett. 112 (2), 223-227 (1993)

MEDLINE 94010250

PUBMED 8405965

COMMENT EC=5.4.99.5.

FEATURES Location/Qualifiers

source

1..75

/organism="Clostridium pasteurianum"

/db\_xref="taxon:1501"

Region

1..73

/region\_name="Glutaredoxin"

/note="Glutaredoxin"

/db\_xref="CDD:23000"



ORIGIN

1 mikiystptc pwckktkeyl ksknidfvdv nvaddmkere emrslskqsg vpvinidgni  
 61 ivgfnkaeid kliek

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

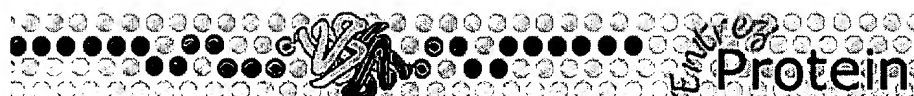
Limits

☐ 1: [AAB20233](#). Reports rubredoxin, Rd [...[gi:238348](#)] [BLink, Domains, Links](#)

LOCUS            AAB20233                            53 aa                            linear    BCT 07-MAY-1993  
 DEFINITION    rubredoxin, Rd [Pyrococcus furiosus, DSM 3638, Peptide, 53 aa].  
 ACCESSION     AAB20233  
 VERSION       AAB20233.1    GI:238348  
 DBSOURCE      accession [AAB20233.1](#)  
 KEYWORDS       .  
 SOURCE        Pyrococcus furiosus  
                 ORGANISM    Pyrococcus furiosus  
                                 Archaea; Euryarchaeota; Thermococci; Thermococcales;  
                                 Thermococcaceae; Pyrococcus.  
 REFERENCE     1 (residues 1 to 53)  
                 AUTHORS    Blake,P.R., Park,J.B., Bryant,F.O., Aono,S., Magnuson,J.K.,  
                                 Eccleston,E., Howard,J.B., Summers,M.F. and Adams,M.W.  
                 TITLE       Determinants of protein hyperthermostability: purification and  
                                 amino acid sequence of rubredoxin from the hyperthermophilic  
                                 archaeobacterium Pyrococcus furiosus and secondary structure of the  
                                 zinc adduct by NMR  
                 JOURNAL     Biochemistry 30 (45), 10885-10895 (1991)  
                 MEDLINE     [92031546](#)  
                 PUBMED      [1932012](#)  
 REMARK        GenBank staff at the National Library of Medicine created this  
                                 entry [NCBI gibbsq 62795] from the original journal article.  
 COMMENT       Method: direct peptide sequencing.  
 FEATURES       Location/Qualifiers  
                 source            1..53  
                                         /organism="Pyrococcus furiosus"  
                                         /db\_xref="taxon:2261"  
                 Protein            1..53  
                                         /product="rubredoxin"  
                                         /note="Rd"  
                 Region            2..51  
                                         /region\_name="Rubredoxin"  
                                         /note="rubredoxin"  
                                         /db\_xref="CDD:24175"  
 ORIGIN  
                 1 akwvckicgy iydedagdpd ngispgtkfe elpddwvcp i cgapksefek led  
 //

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Bio

Search Protein for:

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: [AAB34046](#). Reports rubredoxin, Rd=sm...[gi:998902]BLink, Domains,  
Links

LOCUS AAB34046 52 aa linear BCT 27-SEP-1995

DEFINITION rubredoxin, Rd=small nonheme iron protein [*Heliobacillus mobilis*, Peptide, 52 aa].

ACCESSION AAB34046

VERSION AAB34046.1 GI:998902

DBSOURCE accession [AAB34046.1](#)

KEYWORDS .

SOURCE *Heliobacillus mobilis*

ORGANISM *Heliobacillus mobilis*  
Bacteria; Firmicutes; Clostridia; Clostridiales; Heliobacteriaceae; *Heliobacillus*.

REFERENCE 1 (residues 1 to 52)

AUTHORS Lee, W.Y., Brune, D.C., LoBrutto, R. and Blankenship, R.E.

TITLE Isolation, characterization, and primary structure of rubredoxin from the photosynthetic bacterium, *Heliobacillus mobilis*

JOURNAL Arch. Biochem. Biophys. 318 (1), 80-88 (1995)

MEDLINE [95243660](#)

PUBMED [7726577](#)

REMARK GenBank staff at the National Library of Medicine created this entry [NCBI gibbsq 165993] from the original journal article.

COMMENT Method: direct peptide sequencing.

FEATURES Location/Qualifiers

source 1..52  
/organism="Heliobacillus mobilis"  
/db\_xref="taxon:28064"

Protein 1..52  
/product="rubredoxin"  
/name="small nonheme iron protein"  
/note="Rd"

Region 3..52  
/region\_name="Rubredoxin"  
/note="rubredoxin"  
/db\_xref="CDD:24175"



ORIGIN

1 mkkygclvcg yvydpakgdp dhgiapgtaf edlpadwvcp lcgvsksdefe pl

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

☐ 1: [CAA67880](#). Reports rubredoxin oxydor...[gi:1491677] [BLink, Domains, Links](#)

LOCUS CAA67880 126 aa linear BCT 13-DEC-1996  
 DEFINITION rubredoxin oxydoreductase [Desulfovibrio baarsii].  
 ACCESSION CAA67880  
 VERSION CAA67880.1 GI:1491677  
 DBSOURCE embl locus DBRBORUB, accession [X99543.1](#)  
 KEYWORDS .  
 SOURCE Desulfovibrio baarsii  
   ORGANISM [Desulfovibrio baarsii](#)  
           Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
           Desulfovibrionaceae; Desulfovibrio.  
 REFERENCE 1  
   AUTHORS Pianzola,M.J., Soubes,M. and Touati,D.  
   TITLE Overproduction of the rbo gene product from Desulfovibrio species  
           suppresses all deleterious effects of lack of superoxide dismutase  
           in Escherichia coli  
   JOURNAL J. Bacteriol. 178 (23), 6736-6742 (1996)  
   MEDLINE [97113430](#)  
   PUBMED [8955290](#)  
 REFERENCE 2  
   AUTHORS Touati,D.  
   TITLE Direct Submission  
   JOURNAL Submitted (24-JUL-1996) D. Touati, Institut Jacques Monod,  
           Microbiologie, Unversite Paris 7, Tour 43, 2 Place Jussieu, 75251  
           Paris Cedex 05, FRANCE  
 REMARK revised by [3]  
 REFERENCE 3 (residues 1 to 126)  
   AUTHORS Touati,D.  
   TITLE Direct Submission  
   JOURNAL Submitted (20-NOV-1996) D. Touati, Institut Jacques Monod,  
           Microbiologie, Unversite Paris 7, Tour 43, 2 Place Jussieu, 75251  
           Paris Cedex 05, FRANCE  
 FEATURES Location/Qualifiers  
   source 1..126  
           /organism="Desulfovibrio baarsii"  
           /strain="DSM 2075"  
           /db\_xref="taxon:887"  
   Protein 1..126  
           /product="rubredoxin oxydoreductase"  
   Region 2..37  
           /region\_name="Desulfoferrodoxin, N-terminal domain"  
           /note="Desulfoferrod\_N"  
           /db\_xref="CDD:[26510](#)"  
   Region 41..125  
           /region\_name="Desulfoferrodoxin"  
           /note="Desulfoferrodox"  
           /db\_xref="CDD:[25838](#)"  
   CDS 1..126  
           /gene="rbo"

```
/coded_by="X99543.1:185..565"  
/transl_table=11  
/db_xref="GOA:Q46495"  
/db_xref="UniProt/Swiss-Prot:Q46495"
```


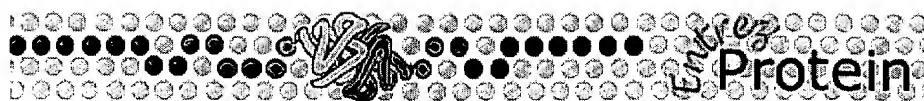
ORIGIN

```
1 mperlqvykc evcgnivevl nggigelvcc nqdmklmsen tvdaakekhv pviekidggy  
61 kvkvgavahp meekhyiqwi elladdkcyt qflkpggape avflieaakv vareycnihg  
121 hwkaen
```

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

Limits   Preview/Index   History   Clipboard   Details

default   File

☐ 1: [CAA67881](#). Reports rubredoxin [Desul...[gi:1491678]

[BLink, Domains, Links](#)

LOCUS CAA67881 53 aa linear BCT 13-DEC-1996  
 DEFINITION rubredoxin [Desulfovibrio baarsii].  
 ACCESSION CAA67881  
 VERSION CAA67881.1 GI:1491678  
 DBSOURCE embl locus DBRBORUB, accession [X99543.1](#)  
 KEYWORDS .  
 SOURCE Desulfovibrio baarsii  
 ORGANISM [Desulfovibrio baarsii](#)  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1  
 AUTHORS Pianzzola,M.J., Soubes,M. and Touati,D.  
 TITLE Overproduction of the rbo gene product from Desulfovibrio species suppresses all deleterious effects of lack of superoxide dismutase in Escherichia coli  
 JOURNAL J. Bacteriol. 178 (23), 6736-6742 (1996)  
 MEDLINE [97113430](#)  
 PUBMED [8955290](#)

REFERENCE 2  
 AUTHORS Touati,D.  
 TITLE Direct Submission  
 JOURNAL Submitted (24-JUL-1996) D. Touati, Institut Jacques Monod, Microbiologie, Unversite Paris 7, Tour 43, 2 Place Jussieu, 75251 Paris Cedex 05, FRANCE  
 REMARK revised by [3]

REFERENCE 3 (residues 1 to 53)  
 AUTHORS Touati,D.  
 TITLE Direct Submission  
 JOURNAL Submitted (20-NOV-1996) D. Touati, Institut Jacques Monod, Microbiologie, Unversite Paris 7, Tour 43, 2 Place Jussieu, 75251 Paris Cedex 05, FRANCE

FEATURES Location/Qualifiers  
 source 1..53  
 /organism="Desulfovibrio baarsii"  
 /strain="DSM 2075"  
 /db\_xref="taxon:887"  
Protein 1..53  
 /product="rubredoxin"  
Region 3..50  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:[24175](#)"  
CDS 1..53  
 /gene="rub"  
 /coded\_by="X99543.1:615..776"  
 /transl\_table=11  
 /db\_xref="GOA:[Q46496](#)"  
 /db\_xref="UniProt/TrEMBL:[Q46496](#)"




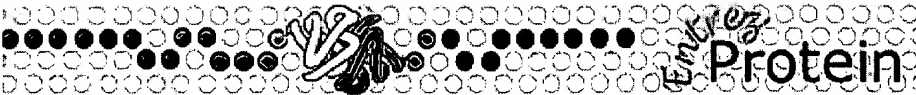
ORIGIN

1 mkkyvcgv cv yeydpakgdp dngvapgtaf edlpadwscp vcgaskdqfe aqa

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Boo

Search  for

Limits Preview/Index History Clipboard Details

☐ 1: [CAA72620](#). Reports rubredoxin [Clost...[gi:1894774]

BLink, Domains, Links

LOCUS CAA72620 53 aa linear BCT 17-MAR-1997

DEFINITION rubredoxin [Clostridium diolis].

ACCESSION CAA72620

VERSION CAA72620.1 GI:1894774

DBSOURCE embl locus CBRUBRDOX, accession [Y11875.1](#)

KEYWORDS .

SOURCE Clostridium diolis

ORGANISM [Clostridium diolis](#)

Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium.

REFERENCE 1

AUTHORS Gerard, P.

JOURNAL Unpublished

REFERENCE 2 (residues 1 to 53)

AUTHORS Gerard, P.

TITLE Direct Submission

JOURNAL Submitted (13-MAR-1997) P. Gerard, Universite Henri Poincare, Laboratoire de Chimie Biologique, Facult des Sciences, 54506 Vandoeuvre-Les-Nancy Cdex, BP 239, FRANCE

FEATURES Location/Qualifiers

source

1..53

/organism="Clostridium diolis"

/strain="DSM5431"

/db\_xref="taxon:223919"

Protein

1..53

/product="rubredoxin"

Region

3..51

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

CDS

1..53

/coded\_by="Y11875.1:422..583"

/transl\_table=11

/db\_xref="GOA:P96745"

/db\_xref="UniProt/TrEMBL:P96745"

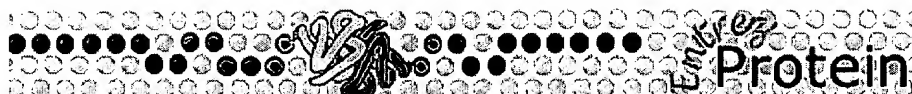
ORIGIN

1 mekyictvcg yiydeaagd dngvapgtkf edipddwvcp lcgvpksdfe keq

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein  for 

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: [AAB50346](#). Reports rubredoxin oxidor...[gi:1905953]BLink, Domains,  
Links

LOCUS AAB50346 393 aa linear BCT 25-MAR-1997  
 DEFINITION rubredoxin oxidoreductase homolog.  
 ACCESSION AAB50346  
 VERSION AAB50346.1 GI:1905953  
 DBSOURCE locus CAU52368 accession [U52368.1](#)  
 KEYWORDS .  
 SOURCE Clostridium acetobutylicum  
 ORGANISM [Clostridium acetobutylicum](#)  
 Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.  
 REFERENCE 1 (residues 1 to 393)  
 AUTHORS Belouski,E., Gui,L. and Bennett,G.N.  
 TITLE Direct Submission  
 JOURNAL Submitted (25-MAR-1996) Ed Belouski, Biochemistry and Cell Biology,  
 Rice University, 6100 S. Main, Houston, TX 77005-1892, USA  
 FEATURES Location/Qualifiers  
 source 1..393  
 /organism="Clostridium acetobutylicum"  
 /strain="ATCC 824"  
 /db\_xref="taxon:1488"  
Protein 1..393  
 /product="rubredoxin oxidoreductase homolog"  
Region <1..392  
 /region\_name="Primosomal protein N' (replication factor Y)  
 - superfamily II helicase [DNA replication, recombination,  
 and repair]"  
 /note="PriA"  
 /db\_xref="CDD:10916"  
CDS 1..393  
 /coded\_by="U52368.1:500..1681"  
 /note="ORFrubr; similar to primosomal protein n' in  
 Haemophilus influenzae, Swiss-Prot Accession Number  
 P44647"  
 /transl\_table=11

## ORIGIN

```

1 mksaiedcil vlgsatpave tyyktlkedy dlitlknrad galmpevsvv dmreelrenn
61 ksifsralye siletlerke qiilflnrrg ystfvscrkc gyvfckcnkd vsmtynhnnkg
121 ylichycgst gripsvcpkc gskyvkyfga gteriereik rlfpnartvr mdrdttvkdd
181 syeryimasr tkgydiligt qmiakaldfk dvtlvgvva dltlnlpdyr agertfqllc
241 qvsgragrgrs kkgkvviqty spesyairfs stndyrsffk eeiklrhdmd yppfnkl111
301 nfsskneqll ikniqivgdf lknkakeysn iyvlgpcpsp iskineayrw qivlkgdity
361 evacfikksi yellkklynn irvsldinpl sll


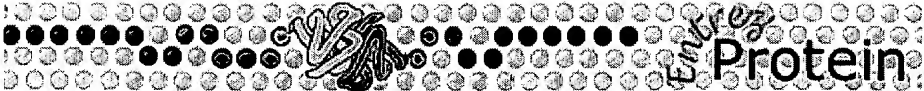
```

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

**THIS PAGE BLANK (USPTO)**

Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Boo

Search **Protein** for

Limits Preview/Index History Clipboard Details

default Show: 20  File

☐ 1: 1RDG. Reports Chain , Rubredox...[gi:230285] BLink, Domains, Links

LOCUS 1RDG 53 aa linear BCT 04-SEP-1998

DEFINITION Rubredoxin.

ACCESSION 1RDG

VERSION 1RDG GI:230285

DBSOURCE pdb: molecule 1RDG, chain 32, release Mar 17, 1988;  
deposition: Mar 17, 1988;  
class: Electron Transfer(Iron-Sulfur Protein);  
source: (Desulfovibrio gigas);  
Exp. method: X-Ray Diffraction.

KEYWORDS .

SOURCE Desulfovibrio gigas

ORGANISM Desulfovibrio gigas  
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1 (residues 1 to 53)

AUTHORS Bruschi,M.

TITLE The amino acid sequence of rubredoxin from the sulfate reducing  
bacterium, Desulfovibrio gigas

JOURNAL Biochem. Biophys. Res. Commun. 70 (2), 615-621 (1976)

MEDLINE [76231572](#)

PUBMED [938515](#)

REFERENCE 2 (residues 1 to 53)

AUTHORS Pierrot,M., Haser,R., Frey,M., Bruschi,M., le Gall,J., Sieker,L.C.  
and Jensen,L.H.

TITLE Some comparisons between two crystallized anaerobic bacterial  
ruberdoxins from Desulfovibrio gigas and D. vulgaris

JOURNAL J. Mol. Biol. 107 (2), 179-182 (1976)

MEDLINE [77074146](#)

PUBMED [1003467](#)

REFERENCE 3 (residues 1 to 53)

AUTHORS Frey,M., Sieker,L., Payan,F., Haser,R., Bruschi,M., Pepe,G. and  
LeGall,J.

TITLE Rubredoxin from Desulfovibrio gigas. A molecular model of the  
oxidized form at 1.4 A resolution

JOURNAL J. Mol. Biol. 197 (3), 525-541 (1987)

MEDLINE [88155649](#)

PUBMED [3441010](#)

REFERENCE 4 (residues 1 to 53)

AUTHORS Frey,M., Sieker,L.C. and Payan,F.

TITLE Direct Submission

JOURNAL Submitted (17-MAR-1988)

COMMENT Revision History:  
JAN 15 91 Typographical  
APR 19 89 Initial Entry.



FEATURES Location/Qualifiers

source 1..53  
/organism="Desulfovibrio gigas"  
/db\_xref="taxon:879"

```
SecStr          4..8
                  /sec_str_type="sheet"
                  /note="strand 1"
Region         5..52
                  /region_name="Rubredoxin"
                  /note="rubredoxin"
                  /db_xref="CDD:24175"
Het           join(bond(7),bond(10),bond(40),bond(43),bond(43),bond(43),
                  bond(43),bond(43),bond(43),bond(43),bond(43),bond(43),
                  bond(43),bond(43),bond(43),bond(43),bond(43))
                  /heterogen="( FE, 53 ) Fe +++"
SecStr         12..15
                  /sec_str_type="sheet"
                  /note="strand 2"
SecStr         17..22
                  /sec_str_type="sheet"
                  /note="strand 3"
SecStr         23..28
                  /sec_str_type="sheet"
                  /note="strand 4"
SecStr         49..53
                  /sec_str_type="sheet"
                  /note="strand 5"
ORIGIN
1 xmdiyvctvc gyeydpakgd pdsgikpgtk fedlpddwac pvcgaskdaf ekq
//
```

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

Show:

☐ 1: [4RXN](#). Reports Chain , Rubredox...[gi:231077] [BLink, Domains, Links](#)

LOCUS 4RXN 54 aa linear BCT 07-OCT-1998  
 DEFINITION Rubredoxin (Oxidized, Fe(III)) (Unconstrained Model).  
 ACCESSION 4RXN  
 VERSION 4RXN GI:231077  
 DBSOURCE pdb: molecule 4RXN, chain 32, release Oct 15, 1984;  
 deposition: Oct 15, 1984;  
 class: Electron Transfer(Iron-Sulfur Protein);  
 source: (Clostridium pasteurianum);  
 Exp. method: X-Ray Diffraction.

KEYWORDS .  
 SOURCE Clostridium pasteurianum  
 ORGANISM Clostridium pasteurianum  
 Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 54)  
 AUTHORS Herriott,J.R., Sieker,L.C., Jensen,L.H. and Lovenberg,W.  
 TITLE Structure of rubredoxin: an x-ray study to 2.5 A resolution  
 JOURNAL J. Mol. Biol. 50 (2), 391-406 (1970)  
 MEDLINE [71027253](#)  
 PUBMED [5476919](#)

REFERENCE 2 (residues 1 to 54)  
 AUTHORS McCarthy,K.F.  
 TITLE The Primary Structure Of Clostridium Pasteurianum Rubredoxin  
 JOURNAL Diss.Abstr.B, Ph.D.Thesis, George Washington University 33, 1436  
 (1972)

REFERENCE 3 (residues 1 to 54)  
 AUTHORS Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.  
 TITLE The structure of a non-heme iron protein: rubredoxin at 1.5  
 Angstrom resolution  
 JOURNAL Cold Spring Harb. Symp. Quant. Biol. 36, 359-367 (1972)  
 MEDLINE [73041148](#)  
 PUBMED [4508149](#)

REFERENCE 4 (residues 1 to 54)  
 AUTHORS Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.  
 TITLE Refinement Of The Model Of A Protein. Rubredoxin At 1.5 Angstroms  
 Resolution  
 JOURNAL Acta Crystallogr.,Sect.B 29, 943 (1973)

REFERENCE 5 (residues 1 to 54)  
 AUTHORS Herriott,J.R., Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.  
 TITLE Sequence of rubredoxin by x-ray diffraction  
 JOURNAL J. Mol. Biol. 80 (3), 423-432 (1973)  
 MEDLINE [74053319](#)  
 PUBMED [4762562](#)


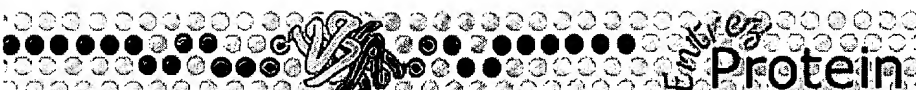
REFERENCE 6 (residues 1 to 54)  
 AUTHORS Watenpaugh,K.D., Margulis,T.N., Sieker,L.C. and Jensen,L.H.  
 TITLE Water structure in a protein crystal: rubredoxin at 1.2 A  
 resolution  
 JOURNAL J. Mol. Biol. 122 (2), 175-190 (1978)

MEDLINE 78244644  
PUBMED 682189  
REFERENCE 7 (residues 1 to 54)  
AUTHORS Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.  
TITLE The structure of rubredoxin at 1.2 A resolution  
JOURNAL J. Mol. Biol. 131 (3), 509-522 (1979)  
MEDLINE 80074980  
PUBMED 513127  
REFERENCE 8 (residues 1 to 54)  
AUTHORS Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.  
TITLE Crystallographic refinement of rubredoxin at 1 x 2 A degrees resolution  
JOURNAL J. Mol. Biol. 138 (3), 615-633 (1980)  
MEDLINE 81009589  
PUBMED 7411618  
REFERENCE 9 (residues 1 to 54)  
AUTHORS Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.  
TITLE Direct Submission  
JOURNAL Submitted (15-OCT-1984)  
COMMENT Revision History:  
OCT 15 91 Typographical  
APR 1 85 Initial Entry.  
FEATURES Location/Qualifiers  
source 1..54  
/organism="Clostridium pasteurianum"  
/db\_xref="taxon:1501"  
Region 3..52  
/region\_name="Rubredoxin"  
/note="rubredoxin"  
/db\_xref="CDD:24175"  
SecStr 3..8  
/sec\_str\_type="sheet"  
/note="strand 1"  
Het join(bond(6),bond(9),bond(39),bond(42),bond(42),bond(42),  
bond(42),bond(42),bond(42),bond(42),bond(42))  
/heterogen="( FE, 1 ) Fe(Iii) Atom"  
SecStr 9..14  
/sec\_str\_type="sheet"  
/note="strand 2"  
SecStr 48..52  
/sec\_str\_type="sheet"  
/note="strand 3"  
ORIGIN  
1 mkkylctvcg yiydpedgdp ddgvnpgtdf kdipddwvcp lcgvgkdefe evee  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Boo

Search Protein  for

Limits Preview/Index History Clipboard Details

default   File

☐ 1: 5RXN. Reports Chain , Rubredox...[gi:231175] BLink, Domains, Links

LOCUS 5RXN 54 aa linear BCT 07-OCT-1998  
DEFINITION Rubredoxin (Oxidized, Fe(III)) (Constrained Model).  
ACCESSION 5RXN  
VERSION 5RXN GI:231175  
DBSOURCE pdb: molecule 5RXN, chain 32, release Oct 15, 1984;  
deposition: Oct 15, 1984;  
class: Electron Transfer(Iron-Sulfur Protein);  
source: (Clostridium pasteurianum);  
Exp. method: X-Ray Diffraction.

KEYWORDS .  
SOURCE Clostridium pasteurianum  
ORGANISM Clostridium pasteurianum  
Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
Clostridium.

REFERENCE 1 (residues 1 to 54)  
AUTHORS Herriott,J.R., Sieker,L.C., Jensen,L.H. and Lovenberg,W.  
TITLE Structure of rubredoxin: an x-ray study to 2.5 A resolution  
JOURNAL J. Mol. Biol. 50 (2), 391-406 (1970)  
MEDLINE 71027253  
PUBMED 5476919

REFERENCE 2 (residues 1 to 54)  
AUTHORS McCarthy,K.F.  
TITLE The Primary Structure Of Clostridium Pasteurianum Rubredoxin  
JOURNAL Diss.Abstr.B, Ph.D.Thesis, George Washington University 33, 1436  
(1972)

REFERENCE 3 (residues 1 to 54)  
AUTHORS Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.  
TITLE The structure of a non-heme iron protein: rubredoxin at 1.5  
Angstrom resolution  
JOURNAL Cold Spring Harb. Symp. Quant. Biol. 36, 359-367 (1972)  
MEDLINE 73041148  
PUBMED 4508149

REFERENCE 4 (residues 1 to 54)  
AUTHORS Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.  
TITLE Refinement Of The Model Of A Protein. Rubredoxin At 1.5 Angstroms  
Resolution  
JOURNAL Acta Crystallogr.,Sect.B 29, 943 (1973)

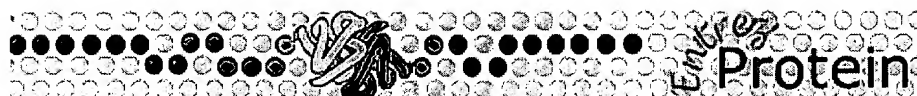
REFERENCE 5 (residues 1 to 54)  
AUTHORS Herriott,J.R., Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.  
TITLE Sequence of rubredoxin by x-ray diffraction  
JOURNAL J. Mol. Biol. 80 (3), 423-432 (1973)  
MEDLINE 74053319  
PUBMED 4762562

REFERENCE 6 (residues 1 to 54)  
AUTHORS Watenpaugh,K.D., Margulis,T.N., Sieker,L.C. and Jensen,L.H.  
TITLE Water structure in a protein crystal: rubredoxin at 1.2 A  
resolution  
JOURNAL J. Mol. Biol. 122 (2), 175-190 (1978)

MEDLINE [78244644](#)  
 PUBMED [682189](#)  
 REFERENCE 7 (residues 1 to 54)  
 AUTHORS Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.  
 TITLE The structure of rubredoxin at 1.2 A resolution  
 JOURNAL J. Mol. Biol. 131 (3), 509-522 (1979)  
 MEDLINE [80074980](#)  
 PUBMED [513127](#)  
 REFERENCE 8 (residues 1 to 54)  
 AUTHORS Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.  
 TITLE Crystallographic refinement of rubredoxin at 1 x 2 A degrees resolution  
 JOURNAL J. Mol. Biol. 138 (3), 615-633 (1980)  
 MEDLINE [81009589](#)  
 PUBMED [7411618](#)  
 REFERENCE 9 (residues 1 to 54)  
 AUTHORS Watenpaugh,K.D.  
 TITLE Combined Crystallographic Refinement And Energy Minimization Of Rubredoxin At 1.2 Angstrom Resolution  
 JOURNAL Unpublished  
 REFERENCE 10 (residues 1 to 54)  
 AUTHORS Watenpaugh,K.D.  
 TITLE Direct Submission  
 JOURNAL Submitted (15-OCT-1984)  
 COMMENT Revision History:  
 OCT 15 91 Typographical  
 APR 1 85 Initial Entry.  
 FEATURES Location/Qualifiers  
     source 1..54  
         /organism="Clostridium pasteurianum"  
         /db\_xref="taxon:1501"  
     Region 3..52  
         /region\_name="Rubredoxin"  
         /note="rubredoxin"  
         /db\_xref="CDD:24175"  
     SecStr 3..8  
         /sec\_str\_type="sheet"  
         /note="strand 1"  
     Het join(bond(6),bond(9),bond(39),bond(42),bond(42),bond(42),bond(42))  
         /heterogen="( FE, 1 ) Fe(Iii) Atom"  
     SecStr 9..14  
         /sec\_str\_type="sheet"  
         /note="strand 2"  
     SecStr 16..19  
         /sec\_str\_type="sheet"  
         /note="strand 3"  
     SecStr 24..27  
         /sec\_str\_type="sheet"  
         /note="strand 4"  
     SecStr 48..52  
         /sec\_str\_type="sheet"  
         /note="strand 5"  
 ORIGIN  
     1 mkkytctvcg yiydpedgdp ddgvnpgtdf kdipddwvcp lcgvgkdefe evee  
 //

Nov 8 2004 13:44:10

**THIS PAGE BLANK (USPTO)**



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 6RXN. Reports Chain, Rubredox...[gi:231232]

 BLink, Domains,  
Links

LOCUS 6RXN 46 aa linear BCT 07-OCT-1998  
 DEFINITION Rubredoxin.  
 ACCESSION 6RXN  
 VERSION 6RXN GI:231232  
 DBSOURCE pdb: molecule 6RXN, chain 32, release Jan 16, 1990;  
 deposition: Jan 16, 1990;  
 class: Electron Transfer(Iron-Sulfur Protein);  
 source: (Desulfovibrio desulfuricans, Strain 27774);  
 Exp. method: X-Ray Diffraction.

KEYWORDS .  
 SOURCE Desulfovibrio desulfuricans  
 ORGANISM Desulfovibrio desulfuricans  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1 (residues 1 to 46)  
 AUTHORS Sieker,L.C., Jensen,L.H., Prickril,B.C. and LeGall,J.  
 TITLE Crystallographic study of rubredoxin from the bacterium  
 Desulfovibrio desulfuricans strain 27774  
 JOURNAL J. Mol. Biol. 171 (1), 101-103 (1983)  
 MEDLINE 84064867  
 PUBMED 6644818

REFERENCE 2 (residues 1 to 46)  
 AUTHORS Sieker,L.C., Stenkamp,R.E., Jensen,L.H., Prickril,B. and LeGall,J.  
 TITLE Structure of rubredoxin from the bacterium Desulfovibrio  
 desulfuricans  
 JOURNAL FEBS Lett. 208 (1), 73-76 (1986)  
 MEDLINE 87030959  
 PUBMED 3770211

REFERENCE 3 (residues 1 to 46)  
 AUTHORS Stenkamp,R.E., Sieker,L.C. and Jensen,L.H.  
 TITLE The Structure Of Rubredoxin From Desulfovibrio desulfuricans  
 JOURNAL Unpublished

REFERENCE 4 (residues 1 to 46)  
 AUTHORS Stenkamp,R.E., Sieker,L.C. and Jensen,L.H.  
 TITLE Direct Submission  
 JOURNAL Submitted (16-JAN-1990)

COMMENT Revision History:  
 JUL 15 91 Coordinates  
 JAN 15 91 Initial Entry.

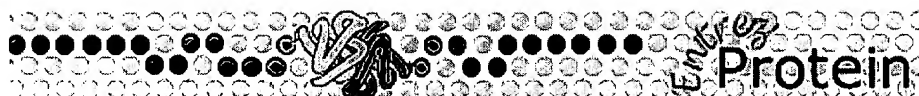
FEATURES Location/Qualifiers  
 source 1..46  
 /organism="Desulfovibrio desulfuricans"  
 /db\_xref="taxon:876"  
 Region 4..45  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 SecStr 4..8

```

                                /sec_str_type="sheet"
                                /note="strand 1"
Het      join(bond(7),bond(10),bond(33),bond(36),bond(36),bond(36),
                                bond(36),bond(36),bond(36),bond(36),bond(36),bond(36),
                                bond(36),bond(36),bond(36),bond(36),bond(36),bond(36),
                                bond(36),bond(36),bond(36),bond(36),bond(36),bond(36),
                                bond(36),bond(36),bond(36),bond(36),bond(36),bond(36),
                                bond(36),bond(36))
                                /heterogen="( FE, 53 ) Iron"
SecStr    12..15
                                /sec_str_type="sheet"
                                /note="strand 2"
SecStr    42..46
                                /sec_str_type="sheet"
                                /note="strand 3"
ORIGIN
      1 xmqkyvcnvc gyeydpaehd nvpfdqlpdd wccpvcgvsk dqfspa
//
```

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 7RXN. Reports Chain , Rubredox...[gi:231271]

 BLink, Domains,  
Links

LOCUS 7RXN 52 aa linear BCT 07-OCT-1998  
 DEFINITION Rubredoxin.  
 ACCESSION 7RXN  
 VERSION 7RXN GI:231271  
 DBSOURCE pdb: molecule 7RXN, chain 32, release May 11, 1990;  
 deposition: May 11, 1990;  
 class: Electron Transfer(Iron-Sulfur Protein);  
 source: (Desulfovibrio vulgaris);  
 Exp. method: X-Ray Diffraction.

KEYWORDS .  
 SOURCE Desulfovibrio vulgaris  
 ORGANISM Desulfovibrio vulgaris  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1 (residues 1 to 52)  
 AUTHORS Adman,E.T., Sieker,L.C., Jensen,L.H., Bruschi,M. and Le Gall,J.  
 TITLE A structural model of rubredoxin from Desulfovibrio vulgaris at 2 A resolution  
 JOURNAL J. Mol. Biol. 112 (1), 113-120 (1977)  
 MEDLINE 77230456  
 PUBMED 881725

REFERENCE 2 (residues 1 to 52)  
 AUTHORS Adman,E.T. and Jensen,L.H.  
 TITLE Progress On Refinement Of Rubredoxin (D. Vulgaris) At 1.5 Angstroms  
 JOURNAL Am.Cryst.Assoc.,Abstr.Papers (Winter Meeting) 6, 65 (1979)

REFERENCE 3 (residues 1 to 52)  
 AUTHORS Adman,E.T., Sieker,L.C. and Jensen,L.H.  
 TITLE Structure of rubredoxin from Desulfovibrio vulgaris at 1.5 A resolution  
 JOURNAL J. Mol. Biol. 217 (2), 337-352 (1991)  
 MEDLINE 91124457  
 PUBMED 1992166

REFERENCE 4 (residues 1 to 52)  
 AUTHORS Adman,E.T., Sieker,L.C. and Jensen,L.H.  
 TITLE Direct Submission  
 JOURNAL Submitted (11-MAY-1990)

COMMENT Revision History:  
 JAN 15 93 Typographical  
 JUL 15 91 Initial Entry.

FEATURES Location/Qualifiers  
 source 1..52  
 /organism="Desulfovibrio vulgaris".  
 /db\_xref="taxon:881"  
 Region 3..50  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 SecStr 3..7

```

                                /sec_str_type="sheet"
                                /note="strand 1"
Het      join(bond(6),bond(9),bond(39),bond(42))
                                /heterogen="( FE, 53 ) Fe(Iii) Atom"
SecStr   11..14
                                /sec_str_type="sheet"
                                /note="strand 2"
SecStr   16..19
                                /sec_str_type="sheet"
                                /note="strand 3"
SecStr   24..27
                                /sec_str_type="sheet"
                                /note="strand 4"
SecStr   48..52
                                /sec_str_type="sheet"
                                /note="strand 5"
ORIGIN
      1 mkkyvctvcg yeydpaegdp dngvkpgtsf ddlpadwvcp vcgapksefe aa
//
```

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 1CAA. Reports Chain , Rubredox...[gi:442697]

 BLINK, Domains,  
Links

LOCUS 1CAA 53 aa linear BCT 04-SEP-1998

DEFINITION Rubredoxin (Oxidized).

ACCESSION 1CAA

VERSION 1CAA GI:442697

 DBSOURCE pdb: molecule 1CAA, chain 32, release May 18, 1992;  
 deposition: May 18, 1992;  
 class: Electron Transport;  
 source: (Pyrococcus Furiosus);  
 Exp. method: X-Ray Diffraction.

KEYWORDS .

SOURCE Pyrococcus furiosus

ORGANISM Pyrococcus furiosus

 Archaea; Euryarchaeota; Thermococci; Thermococcales;  
 Thermococcaceae; Pyrococcus.

REFERENCE 1 (residues 1 to 53)

 AUTHORS Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B., Zhou,Z.H., Adams,M.W.  
 and Rees,D.C.

 TITLE X-ray crystal structures of the oxidized and reduced forms of the  
 rubredoxin from the marine hyperthermophilic archaeobacterium  
 Pyrococcus furiosus

JOURNAL Protein Sci. 1 (11), 1494-1507 (1992)

MEDLINE 93271899

PUBMED 1303768

REFERENCE 2 (residues 1 to 53)

 AUTHORS Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B., Zhou,Z.H.,  
 Adams,M.W.W. and Rees,D.C.

TITLE Direct Submission

JOURNAL Submitted (18-MAY-1992)

COMMENT Revision History:

APR 30 94 Typographical

OCT 31 93 Initial Entry.

FEATURES Location/Qualifiers

 source 1..53  
 /organism="Pyrococcus furiosus"  
 /db\_xref="taxon:2261"

 SecStr 1..7  
 /sec\_str\_type="sheet"  
 /note="strand 1"

 Region 2..51  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"

 Het join(bond(5),bond(8),bond(38),bond(41))  
 /heterogen="( FE, 54 ) Fe(Iii) Atom"

 SecStr 8..14  
 /sec\_str\_type="sheet"  
 /note="strand 2"

SecStr 47..51



/sec\_str\_type="sheet"  
/note="strand 3"

ORIGIN

// 1 akwvckicgy iydedagdpd ngispgtkfe elpddwvcp i cgapksefek led

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 1CAD. Reports Chain , Rubredox...[gi:442698]BLink, Domains,  
Links

LOCUS 1CAD 53 aa linear BCT 04-SEP-1998  
 DEFINITION Rubredoxin (Reduced).  
 ACCESSION 1CAD  
 VERSION 1CAD GI:442698  
 DBSOURCE pdb: molecule 1CAD, chain 32, release May 18, 1992;  
 deposition: May 18, 1992;  
 class: Electron Transport;  
 source: (Pyrococcus Furiosus);  
 Exp. method: X-Ray Diffraction.  
 KEYWORDS .  
 SOURCE Pyrococcus furiosus  
 ORGANISM Pyrococcus furiosus  
 Archaea; Euryarchaeota; Thermococci; Thermococcales;  
 Thermococcaceae; Pyrococcus.  
 REFERENCE 1 (residues 1 to 53)  
 AUTHORS Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B., Zhou,Z.H., Adams,M.W.  
 and Rees,D.C.  
 TITLE X-ray crystal structures of the oxidized and reduced forms of the  
 rubredoxin from the marine hyperthermophilic archaeobacterium  
 Pyrococcus furiosus  
 JOURNAL Protein Sci. 1 (11), 1494-1507 (1992)  
 MEDLINE 93271899  
 PUBMED 1303768  
 REFERENCE 2 (residues 1 to 53)  
 AUTHORS Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B., Zhou,Z.H.,  
 Adams,M.W.W. and Rees,D.C.  
 TITLE Direct Submission  
 JOURNAL Submitted (18-MAY-1992)  
 COMMENT Revision History:  
 APR 30 94 Typographical  
 OCT 31 93 Initial Entry.  
 FEATURES Location/Qualifiers  
 source 1..53  
 /organism="Pyrococcus furiosus"  
 /db\_xref="taxon:2261"  
 SecStr 1..6  
 /sec\_str\_type="sheet"  
 /note="strand 1"  
 Region 2..51  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 Het join(bond(5),bond(8),bond(38),bond(41))  
 /heterogen="( FE, 54 ) Fe(Iii) Atom"  
 SecStr 9..14  
 /sec\_str\_type="sheet"  
 /note="strand 2"  
 SecStr 15..18

```
                /sec_str_type="sheet"  
                /note="strand 3"  
    SecStr      23..26  
                /sec_str_type="sheet"  
                /note="strand 4"  
    SecStr      47..51  
                /sec_str_type="sheet"  
                /note="strand 5"  
ORIGIN  
      1 akwvckicgy iydedagdpd ngispgtkfe elpddwvcpi cgapksefek led  
//
```

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 1ZRP. Reports Chain , Rubredox...[gi:443341]

 BLINK, Domains,  
Links

LOCUS 1ZRP 53 aa linear BCT 04-SEP-1998  
 DEFINITION Rubredoxin (Zn-Substituted) (Nmr, 40 Structures).  
 ACCESSION 1ZRP  
 VERSION 1ZRP GI:443341  
 DBSOURCE pdb: molecule 1ZRP, chain 32, release Jul 10, 1992;  
 deposition: Jul 10, 1992;  
 class: Electron Transport;  
 source: (Pyrococcus Furiosus);  
 Exp. method: Nmr.

KEYWORDS .  
 SOURCE Pyrococcus furiosus  
 ORGANISM Pyrococcus furiosus  
 Archaea; Euryarchaeota; Thermococci; Thermococcales;  
 Thermococcaceae; Pyrococcus.

REFERENCE 1 (residues 1 to 53)  
 AUTHORS Blake,P.R., Park,J.B., Bryant,F.O., Aono,S., Magnuson,J.K.,  
 Eccleston,E., Howard,J.B., Summers,M.F. and Adams,M.W.  
 TITLE Determinants of protein hyperthermostability: purification and  
 amino acid sequence of rubredoxin from the hyperthermophilic  
 archaeobacterium Pyrococcus furiosus and secondary structure of the  
 zinc adduct by NMR  
 JOURNAL Biochemistry 30 (45), 10885-10895 (1991)  
 MEDLINE 92031546  
 PUBMED 1932012

REFERENCE 2 (residues 1 to 53)  
 AUTHORS Blake,P.R., Park,J.B., Adams,M.W.W. and Summers,M.F.  
 TITLE Novel Observation Of Nh...S(Cys) Hydrogen-Bond-Mediated Scalar  
 Coupling In 113Cd-Substituted Rubredoxin From Pyrococcus Furiosus  
 JOURNAL J.Am.Chem.Soc. 114, 4931 (1992)

REFERENCE 3 (residues 1 to 53)  
 AUTHORS Blake,P.R., Lee,B., Summers,M.F., Adams,M.W., Park,J.B., Zhou,Z.H.  
 and Bax,A.  
 TITLE Quantitative measurement of small through-hydrogen-bond and  
 'through-space' 1H-113Cd and 1H-199Hg J couplings in  
 metal-substituted rubredoxin from Pyrococcus furiosus  
 JOURNAL J. Biomol. NMR 2 (5), 527-533 (1992)  
 MEDLINE 93044392  
 PUBMED 1422158


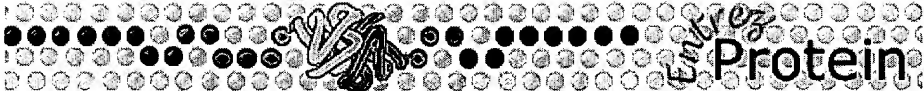
REFERENCE 4 (residues 1 to 53)  
 AUTHORS Blake,P.R., Park,J.B., Zhou,Z.H., Hare,D.R., Adams,M.W. and  
 Summers,M.F.  
 TITLE Solution-state structure by NMR of zinc-substituted rubredoxin from  
 the marine hyperthermophilic archaeobacterium Pyrococcus furiosus  
 JOURNAL Protein Sci. 1 (11), 1508-1521 (1992)  
 MEDLINE 93271900  
 PUBMED 1303769

REFERENCE 5 (residues 1 to 53)  
 AUTHORS Blake,P.R., Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B.,

Hare,D.R., Adams,M.W., Rees,D.C. and Summers,M.F.  
TITLE Comparison of the X-ray structure of native rubredoxin from  
Pyrococcus furiosus with the NMR structure of the zinc-substituted  
protein  
JOURNAL Protein Sci. 1 (11), 1522-1525 (1992)  
MEDLINE 93271901  
PUBMED 1303770  
REFERENCE 6 (residues 1 to 53)  
AUTHORS Blake,P.R., Park,J.B., Zhou,Z.H., Hare,D.R., Adams,M.W.W. and  
Summers,M.F.  
TITLE Direct Submission  
JOURNAL Submitted (10-JUL-1992)  
COMMENT Revision History:  
OCT 31 93 Initial Entry.  
FEATURES Location/Qualifiers  
source 1..53  
/organism="Pyrococcus furiosus"  
/db\_xref="taxon:2261"  
SecStr 1..6  
/sec\_str\_type="sheet"  
/note="strand 1"  
Region 2..51  
/region\_name="Rubredoxin"  
/note="rubredoxin"  
/db\_xref="CDD:24175"  
Het join(bond(5),bond(8),bond(38),bond(41))  
/heterogen="( ZN, 54 ) Zinc"  
SecStr 9..14  
/sec\_str\_type="sheet"  
/note="strand 2"  
SecStr 47..51  
/sec\_str\_type="sheet"  
/note="strand 3"  
ORIGIN  
1 akwvckicgy iydedagdpd ngispgtkfe elpddwvcpj cgapksefek led  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)
[PubMed](#)
[Nucleotide](#)
[Protein](#)
[Genome](#)
[Structure](#)
[PMC](#)
[Taxonomy](#)
[Boo](#)

Search  for

Limits

☐ 1: [8RXNA](#). Reports Chain A, Rubredox...[gi:443567] BLink, Domains, Links

LOCUS 8RXN\_A 52 aa linear BCT 07-OCT-1998  
 DEFINITION Chain A, Rubredoxin.  
 ACCESSION 8RXN\_A  
 VERSION 8RXN\_A GI:443567  
 DBSOURCE pdb: molecule 8RXN, chain 65, release Aug 26, 1991;  
 deposition: Aug 26, 1991;  
 class: Electron Transport(Iron);  
 source: (Desulfovibrio Vulgaris);  
 Exp. method: X-Ray Diffraction.

KEYWORDS .  
 SOURCE Desulfovibrio vulgaris  
 ORGANISM Desulfovibrio vulgaris  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1 (residues 1 to 52)  
 AUTHORS Dauter,Z., Sieker,L.C. and Wilson,K.S.  
 TITLE Refinement of rubredoxin from Desulfovibrio vulgaris at 1.0 A with  
 and without restraints  
 JOURNAL Acta Crystallogr., B 48 (Pt 1), 42-59 (1992)  
 MEDLINE [92313601](#)  
 PUBMED [1616692](#)

REFERENCE 2 (residues 1 to 52)  
 AUTHORS Dauter,Z., Sieker,L. and Wilson,K.  
 TITLE Direct Submission  
 JOURNAL Submitted (26-AUG-1991)

COMMENT Revision History:  
 OCT 31 93 Initial Entry.

FEATURES Location/Qualifiers  
     source 1..52  
         /organism="Desulfovibrio vulgaris"  
         /db\_xref="taxon:881"  
     Region 3..50  
         /region\_name="Rubredoxin"  
         /note="rubredoxin"  
         /db\_xref="CDD:24175"  
     SecStr 3..7  
         /sec\_str\_type="sheet"  
         /note="strand 1"  
     Het join(bond(6),bond(9),bond(9),bond(9),bond(39),bond(42),  
         bond(42))  
         /heterogen="( FE, 55 ) Fe(II) Ion"  
     SecStr 11..14  
         /sec\_str\_type="sheet"  
         /note="strand 2"  
     SecStr 16..19  
         /sec\_str\_type="sheet"  
         /note="strand 3"  
     SecStr 24..27

SecStr


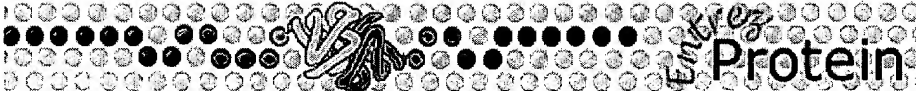
/sec\_str\_type="sheet"  
/note="strand 4"  
48..52  
/sec\_str\_type="sheet"  
/note="strand 5"

ORIGIN

i mkkyvctvcg yeydpaegdp dngvkpgtsf ddlpadwvcp vcgapksefe aa  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Book](#)

Search  for

☐ 1: [1RB9](#). Reports Chain , Rubredox...[gi:4558184] BLink, Domains, Links

LOCUS 1RB9 53 aa linear BCT 21-DEC-1997  
 DEFINITION Rubredoxin From Desulfovibrio Vulgaris Refined Anisotropically At 0.92 Angstroms Resolution.  
 ACCESSION 1RB9  
 VERSION 1RB9 GI:4558184  
 DBSOURCE pdb: molecule 1RB9, chain 32, release Dec 21, 1997;  
 deposition: Dec 21, 1997;  
 class: Iron-Sulfur Protein;  
 source: Mol\_id: 1; Organism\_scientific: Desulfovibrio Vulgaris;  
 Strain: Hildenborough; Expression\_system: Escherichia Coli;  
 Exp. method: X-Ray Diffraction.  
 KEYWORDS .  
 SOURCE Desulfovibrio vulgaris  
 ORGANISM Desulfovibrio vulgaris  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.  
 REFERENCE 1 (residues 1 to 53)  
 AUTHORS Dauter,Z., Butterworth,S., Sieker,L.C., Sheldrick,G. and Wilson,K.S.  
 TITLE Anisotropic Refinement Of Rubredoxin From Desulfovibrio Vulgaris  
 JOURNAL Unpublished  
 REFERENCE 2 (residues 1 to 53)  
 AUTHORS Dauter,Z., Butterworth,S., Sieker,L.C., Sheldrick,G. and Wilson,K.S.  
 TITLE Direct Submission  
 JOURNAL Submitted (21-DEC-1997)  
 COMMENT Revision History:  
 FEB 16 99 Initial Entry.  
 FEATURES Location/Qualifiers  
 source 1..53  
 /organism="Desulfovibrio vulgaris"  
 /db\_xref="taxon:881"  
 Region 4..51  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 SecStr 4..8  
 /sec\_str\_type="sheet"  
 /note="strand 1"  
 Het join(bond(7),bond(10),bond(40),bond(43),bond(43),bond(43),  
 bond(43),bond(43),bond(43),bond(43),bond(43),bond(43),  
 bond(43),bond(43))  
 /heterogen="(FE2, 54 )"  
 SecStr 12..15  
 /sec\_str\_type="sheet"  
 /note="strand 2"  
 SecStr 17..20  
 /sec\_str\_type="sheet"




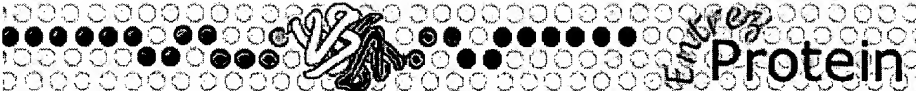
SecStr            /note="strand 3"  
                  25..28  
                  /sec\_str\_type="sheet"  
SecStr            /note="strand 4"  
                  49..53  
                  /sec\_str\_type="sheet"  
                  /note="strand 5"

ORIGIN

1 xmkkyvctvc gyeydpaegd pdngvkgpgts fddlpadwvc pvcgapksef eaa  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

☐ 1: [1BE7](#). Reports Chain , Clostrid...[gi:3659994]
 [BLink, Domains, Links](#)

LOCUS 1BE7 54 aa linear BCT 20-MAY-1998  
 DEFINITION Clostridium Pasteurianum Rubredoxin C42s Mutant.  
 ACCESSION 1BE7  
 VERSION 1BE7 GI:3659994  
 DBSOURCE pdb: molecule 1BE7, chain 32, release May 20, 1998;  
 deposition: May 20, 1998;  
 class: Electron Transport;  
 source: Mol\_id: 1; Organism\_scientific: Clostridium Pasteurianum;  
 Cellular\_location: Cytoplasm; Expression\_system: Escherichia Coli;  
 Expression\_system\_strain: Jm109;  
 Expression\_system\_cellular\_location: Cytoplasm;  
 Expression\_system\_vector\_type: Plasmid; Expression\_system\_plasmid:  
 Pkk223-3; Expression\_system\_gene: Clorub;  
 Exp. method: X-Ray Diffraction.

KEYWORDS .  
 SOURCE Clostridium pasteurianum  
 ORGANISM Clostridium pasteurianum  
 Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 54)  
 AUTHORS Xiao,Z., Lavery,M.J., Ayhan,M., Scrofani,S.D.B., Wilce,M.C.J.,  
 Guss,J.M., Tregloan,P.A., George,G.N. and Wedd,A.G.  
 TITLE The Rubredoxin From Clostridium Pasteurianum: Mutation Of The Iron  
 Cysteiny1 Ligands To Serine. Crystal And Molecular Structures Of  
 The Oxidised And Dithionite-Treated Forms Of The Cys42ser Mutant  
 JOURNAL J.Am.Chem.Soc. 120, 4135 (1998)

REFERENCE 2 (residues 1 to 54)  
 AUTHORS Maher,M., Guss,J.M., Wilce,M. and Wedd,A.G.  
 TITLE Direct Submission  
 JOURNAL Submitted (20-MAY-1998)

COMMENT Revision History:  
 SEP 23 98 Initial Entry.

FEATURES Location/Qualifiers  
 source 1..54  
 /organism="Clostridium pasteurianum"  
 /db\_xref="taxon:1501"  
 Region 3..52  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 SecStr 3..8  
 /sec\_str\_type="sheet"  
 /note="strand 1"  
 Het join(bond(6),bond(9),bond(39),bond(42))  
 /heterogen="( FE, 55 )"  
 SecStr 9..14  
 /sec\_str\_type="sheet"  
 /note="strand 2"

SecStr 16..19  
/sec\_str\_type="sheet"  
/note="strand 3"  
SecStr 24..27  
/sec\_str\_type="sheet"  
/note="strand 4"  
SecStr 48..52  
/sec\_str\_type="sheet"  
/note="strand 5"



ORIGIN

1 mkkytctvcg yiynpedgdp dngvnpgtdf kdipddwvcp lsgvgkdqfe eee

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)
[PubMed](#)
[Nucleotide](#)
[Protein](#)
[Genome](#)
[Structure](#)
[PMC](#)
[Taxonomy](#)
[Boo](#)

Search  for

☐ 1: [1B13A](#). Reports Chain A, Clostrid...[gi:5107456] BLink, Domains, Links

LOCUS 1B13\_A 54 aa linear BCT 26-NOV-1998  
 DEFINITION Chain A, Clostridium Pasteurianum Rubredoxin G10a Mutant.  
 ACCESSION 1B13\_A  
 VERSION 1B13\_A GI:5107456  
 DBSOURCE pdb: molecule 1B13, chain 65, release Nov 26, 1998;  
 deposition: Nov 26, 1998;  
 class: Electron Transport;  
 source: Mol\_id: 1; Organism\_scientific: Clostridium Pasteurianum;  
 Strain: Jm109; Cellular\_location: Cytoplasm; Gene: Clorub;  
 Expression\_system: Escherichia Coli; Expression\_system\_strain:  
 Jm109; Expression\_system\_vector\_type: Plasmid;  
 Expression\_system\_plasmid: Pkk223-3; Expression\_system\_gene:  
 Clorub;  
 Exp. method: X-Ray Diffraction.

KEYWORDS .  
 SOURCE Clostridium pasteurianum  
 ORGANISM Clostridium pasteurianum  
 Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 54)  
 AUTHORS Maher,M.J., Xiao,Z., Wilce,M.C.J., Guss,J.M. and Wedd,A.G.  
 TITLE Rubredoxin From Clostridium Pasteurianum. Structures Of G10a, G43a  
 And G10vg43a Mutant Proteins. Mutation Of Conserved Glycine 10 To  
 Valine Causes The 9-10 Peptide Link To Invert  
 JOURNAL Acta Crystallogr., Sect.D, 962 (1999)  
 REFERENCE 2 (residues 1 to 54)  
 AUTHORS Maher,M.J., Guss,J.M., Wilce,M.C.J. and Wedd,A.G.  
 TITLE Direct Submission  
 JOURNAL Submitted (26-NOV-1998)  
 COMMENT Revision History:  
 MAY 27 99 Initial Entry.

FEATURES Location/Qualifiers  
 source 1..54  
 /organism="Clostridium pasteurianum"  
 /db\_xref="taxon:1501"  
 Region 3..52  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 SecStr 3..8  
 /sec\_str\_type="sheet"  
 /note="strand 1"  
 Het join(bond(6),bond(9),bond(39),bond(42))  
 /heterogen="( FE, 55 )"  
 SecStr 9..14  
 /sec\_str\_type="sheet"  
 /note="strand 2"  
 SecStr 16..19

SecStr            /sec\_str\_type="sheet"  
                  /note="strand 3"  
24..27  
SecStr            /sec\_str\_type="sheet"  
                  /note="strand 4"  
48..52  
SecStr            /sec\_str\_type="sheet"  
                  /note="strand 5"


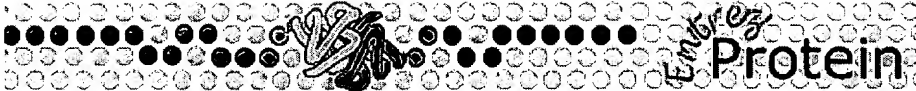
ORIGIN

1 mkkytctvca yiynpedgdp dngvnpgtdf kdipddwvcp lcgvgkdqfe evec

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)
[PubMed](#)
[Nucleotide](#)
[Protein](#)
[Genome](#)
[Structure](#)
[PMC](#)
[Taxonomy](#)
[Boo](#)

Search  for

☐ 1: [1B2JA](#). Reports Chain A, Clostrid...[gi:5107457]

BLink, Domains, Links

LOCUS 1B2J\_A 54 aa linear BCT 27-NOV-1998

DEFINITION Chain A, Clostridium Pasteurianum Rubredoxin G43a Mutant.

ACCESSION 1B2J\_A

VERSION 1B2J\_A GI:5107457

DBSOURCE pdb: molecule 1B2J, chain 65, release Nov 27, 1998;  
 deposition: Nov 27, 1998;  
 class: Electron Transport;  
 source: Mol\_id: 1; Organism\_scientific: Clostridium Pasteurianum;  
 Strain: Jm109; Cellular\_location: Cytoplasm; Gene: Clorub;  
 Expression\_system: Escherichia Coli; Expression\_system\_strain:  
 Jm109; Expression\_system\_vector\_type: Plasmid;  
 Expression\_system\_plasmid: Pkk223-3; Expression\_system\_gene:  
 Clorub;  
 Exp. method: X-Ray Diffraction.

#### KEYWORDS

SOURCE Clostridium pasteurianum

ORGANISM Clostridium pasteurianum

Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 54)

AUTHORS Maher,M.J., Xiao,Z., Wilce,M.C.J., Guss,J.M. and Wedd,A.G.

TITLE Rubredoxin From Clostridium Pasteurianum. Structures Of G10a, G43a  
 And G10vg43a Mutant Proteins. Mutation Of Conserved Glycine 10 To  
 Valine Causes The 9-10 Peptide Link To Invert

JOURNAL Acta Crystallogr., Sect.D, 962 (1999)

REFERENCE 2 (residues 1 to 54)

AUTHORS Maher,M.J., Guss,J.M., Wilce,M.C.J. and Wedd,A.G.

TITLE Direct Submission

JOURNAL Submitted (27-NOV-1998)

COMMENT Revision History:

MAY 27 99 Initial Entry.

FEATURES Location/Qualifiers

source 1..54  
 /organism="Clostridium pasteurianum"  
 /db\_xref="taxon:1501"

Region 3..52  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"

SecStr 3..8  
 /sec\_str\_type="sheet"  
 /note="strand 1"

Het join(bond(6),bond(9),bond(39),bond(42))  
 /heterogen="( FE, 55 )"

SecStr 9..14  
 /sec\_str\_type="sheet"  
 /note="strand 2"

SecStr 16..19

SecStr      /sec\_str\_type="sheet"  
              /note="strand 3"  
24..27  
SecStr      /sec\_str\_type="sheet"  
              /note="strand 4"  
48..52  
SecStr      /sec\_str\_type="sheet"  
              /note="strand 5"



ORIGIN

1 mkkytctvcg yiynpedgdp dngvnpgtdf kdipddwvcp lcavgkdqfe evec

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

☐ 1: [1B2OA](#). Reports Chain A, Clostrid...[gi:5107458] BLink, Domains, Links

LOCUS 1B2O\_A 54 aa linear BCT 30-NOV-1998  
 DEFINITION Chain A, Clostridium Pasteurianum Rubredoxin G10vg43a Mutant.  
 ACCESSION 1B2O\_A  
 VERSION 1B2O\_A GI:5107458  
 DBSOURCE pdb: molecule 1B2O, chain 65, release Nov 30, 1998;  
 deposition: Nov 30, 1998;  
 class: Electron Transport;  
 source: Mol\_id: 1; Organism\_scientific: Clostridium Pasteurianum;  
 Strain: Jm109; Cellular\_location: Cytoplasm; Gene: Clorub;  
 Expression\_system: Escherichia Coli; Expression\_system\_strain:  
 Jm109; Expression\_system\_vector\_type: Plasmid;  
 Expression\_system\_plasmid: Pkk223-3; Expression\_system\_gene:  
 Clorub;  
 Exp. method: X-Ray Diffraction.

KEYWORDS .  
 SOURCE Clostridium pasteurianum  
 ORGANISM Clostridium pasteurianum  
 Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 54)  
 AUTHORS Maher,M.J., Xiao,Z., Wilce,M.C.J., Guss,J.M. and Wedd,A.G.  
 TITLE Rubredoxin From Clostridium Pasteurianum. Structures Of G10a, G43a  
 And G10vg43a Mutant Proteins. Mutation Of Conserved Glycine 10 To  
 Valine Causes The 9-10 Peptide Link To Invert  
 JOURNAL Acta Crystallogr., Sect.D, 962 (1999)  
 REFERENCE 2 (residues 1 to 54)  
 AUTHORS Maher,M.J., Guss,J.M., Wilce,M.C.J. and Wedd,A.G.  
 TITLE Direct Submission  
 JOURNAL Submitted (30-NOV-1998)  
 COMMENT Revision History:  
 MAY 27 99 Initial Entry.

FEATURES Location/Qualifiers  
 source 1..54  
 /organism="Clostridium pasteurianum"  
 /db\_xref="taxon:1501"  
 Region 3..52  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 SecStr 3..7  
 /sec\_str\_type="sheet"  
 /note="strand 1"  
 Het join(bond(6),bond(9),bond(39),bond(42))  
 /heterogen="( FE, 55 )"  
 SecStr 11..14  
 /sec\_str\_type="sheet"  
 /note="strand 2"  
 SecStr 16..19



SecStr            /sec\_str\_type="sheet"  
                  /note="strand 3"  
24..27  
SecStr            /sec\_str\_type="sheet"  
                  /note="strand 4"  
48..52  
                  /sec\_str\_type="sheet"  
                  /note="strand 5"


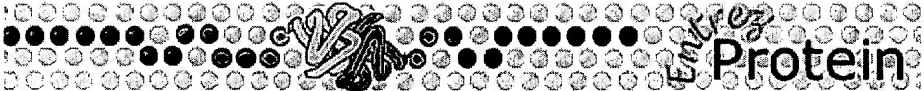
ORIGIN

1 mkkytctvcv yiynpedgdp dngvnpgtdf kdipddwvcp lcavgkdqfe evee

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#) [PubMed](#) [Nucleotide](#) [Protein](#) [Genome](#) [Structure](#) [PMC](#) [Taxonomy](#) [Boo](#)

Search  for

☐ 1: [1B2OB](#). Reports Chain B, Clostrid...[gi:5107459] [BLink, Domains, Links](#)

LOCUS 1B2O\_B 54 aa linear BCT 30-NOV-1998  
 DEFINITION Chain B, Clostridium Pasteurianum Rubredoxin G10vg43a Mutant.  
 ACCESSION 1B2O\_B  
 VERSION 1B2O\_B GI:5107459  
 DBSOURCE pdb: molecule 1B2O, chain 66, release Nov 30, 1998;  
 deposition: Nov 30, 1998;  
 class: Electron Transport;  
 source: Mol\_id: 1; Organism\_scientific: Clostridium Pasteurianum;  
 Strain: Jm109; Cellular\_location: Cytoplasm; Gene: Clorub;  
 Expression\_system: Escherichia Coli; Expression\_system\_strain:  
 Jm109; Expression\_system\_vector\_type: Plasmid;  
 Expression\_system\_plasmid: Pkk223-3; Expression\_system\_gene:  
 Clorub;  
 Exp. method: X-Ray Diffraction.

KEYWORDS .

SOURCE Clostridium pasteurianum  
 ORGANISM [Clostridium pasteurianum](#)  
 Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 54)  
 AUTHORS Maher,M.J., Xiao,Z., Wilce,M.C.J., Guss,J.M. and Wedd,A.G.  
 TITLE Rubredoxin From Clostridium Pasteurianum. Structures Of G10a, G43a  
 And G10vg43a Mutant Proteins. Mutation Of Conserved Glycine 10 To  
 Valine Causes The 9-10 Peptide Link To Invert  
 JOURNAL Acta Crystallogr., Sect.D, 962 (1999)

REFERENCE 2 (residues 1 to 54)  
 AUTHORS Maher,M.J., Guss,J.M., Wilce,M.C.J. and Wedd,A.G.  
 TITLE Direct Submission  
 JOURNAL Submitted (30-NOV-1998)

COMMENT Revision History:  
 MAY 27 99 Initial Entry.

FEATURES Location/Qualifiers  
 source 1..54  
 /organism="Clostridium pasteurianum"  
 /db\_xref="taxon:1501"  
 Region 3..52  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 SecStr 3..7  
 /sec\_str\_type="sheet"  
 /note="strand 6"  
 Het join(bond(6),bond(9),bond(39),bond(42))  
 /heterogen="( FE, 55 )"  
 SecStr 11..14  
 /sec\_str\_type="sheet"  
 /note="strand 7"  
 SecStr 16..21

```
                /sec_str_type="sheet"  
                /note="strand 8"  
    SecStr      22..27  
                /sec_str_type="sheet"  
                /note="strand 9"  
    SecStr      48..52  
                /sec_str_type="sheet"  
                /note="strand 10"  
ORIGIN  
    1 mkkytctvcv yiynpedgdp dngvnpgtdf kdipddwvcp lcavgkdqfe eee  
//
```

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein for:

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 1BFY. Reports Chain, Solution...[gi:5107503]BLink, Domains,  
Links

LOCUS 1BFY 54 aa linear BCT 23-MAY-1998

DEFINITION Solution Structure Of Reduced Clostridium Pasteurianum Rubredoxin, Nmr, 20 Structures.

ACCESSION 1BFY

VERSION 1BFY GI:5107503

DBSOURCE pdb: molecule 1BFY, chain 32, release May 23, 1998;  
deposition: May 23, 1998;  
class: Electron Transport;  
source: Mol\_id: 1; Organism\_scientific: Clostridium Pasteurianum;  
Exp. method: Nmr, 20 Structures.

KEYWORDS .

SOURCE Clostridium pasteurianum

ORGANISM Clostridium pasteurianum  
Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
Clostridium.

REFERENCE 1 (residues 1 to 54)

AUTHORS Bertini, I., Kurtz, D.M. Jr., Eidsness, M.K., Liu, G., Luchinat, C.,  
Rosato, A. and Scott, R.A.

TITLE Solution Structure Of Reduced Clostridium Pasteurianum Rubredoxin

JOURNAL Jbic, J.Biol.Inorg.Chem. 3, 401 (1998)

REFERENCE 2 (residues 1 to 54)

AUTHORS Bertini, I., Kurtz, D.M. Jr., Eidsness, M.K., Liu, G., Luchinat, C.,  
Rosato, A. and Scott, R.A.

TITLE Direct Submission

JOURNAL Submitted (23-MAY-1998)

COMMENT Revision History:  
MAY 25 99 Initial Entry.

FEATURES Location/Qualifiers

source 1..54  
/organism="Clostridium pasteurianum"  
/db\_xref="taxon:1501"

Region 3..52  
/region\_name="Rubredoxin"  
/note="rubredoxin"  
/db\_xref="CDD:24175"

SecStr 3..7  
/sec\_str\_type="sheet"  
/note="strand 1"

Het join(bond(6),bond(9),bond(39),bond(42))  
/heterogen="( FE, 55 )"

SecStr 11..14  
/sec\_str\_type="sheet"  
/note="strand 2"

SecStr 48..52  
/sec\_str\_type="sheet"  
/note="strand 3"


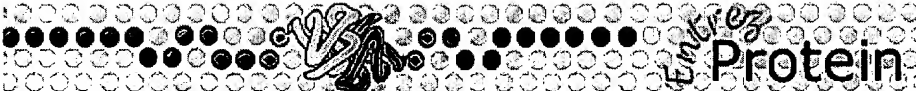
ORIGIN

1 xkkytctvcg yiynpedgdp dngvnpgtdf kdipddwvcp lcgvgkdqfe evee

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

Limits   Preview/Index   History   Clipboard   Details

Show:

☐ 1: [1RDV](#). Reports Chain , Rubredox...[gi:5107680] BLink, Domains, Links

LOCUS 1RDV 52 aa linear BCT 30-SEP-1998  
 DEFINITION Rubredoxin From Desulfovibrio Vulgaris Miyazaki F, Trigonal Crystal Form.  
 ACCESSION 1RDV  
 VERSION 1RDV GI:5107680  
 DBSOURCE pdb: molecule 1RDV, chain 32, release Sep 30, 1998;  
 deposition: Sep 30, 1998;  
 class: Electron Transfer;  
 source: Mol\_id: 1; Organism\_scientific: Desulfovibrio Vulgaris;  
 Strain: Miyazaki F; Other\_details: Iam 12604;  
 Exp. method: X-Ray Diffraction.

KEYWORDS .

SOURCE Desulfovibrio vulgaris  
 ORGANISM Desulfovibrio vulgaris  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1 (residues 1 to 52)  
 AUTHORS Higuchi,Y., Sugiyama,M.S., Morimoto,S., Ogata,Y., Yagi,M. and Yasuoka,T.  
 TITLE Preliminary Crystallographic Study Of Two Crystal Forms Of Rubredoxin From Sulfate-Reducing Bacterium  
 JOURNAL Protein Pept.Lett. 5, 175 (1998)

REFERENCE 2 (residues 1 to 52)  
 AUTHORS Misaki,S., Morimoto,Y., Ogata,M., Yagi,T., Higuchi,Y. and Yasuoka,N.  
 TITLE Structure determination of rubredoxin from Desulfovibrio vulgaris Miyazaki F in two crystal forms  
 JOURNAL Acta Crystallogr D Biol Crystallogr 55 (Pt 2), 408-413 (1999)  
 MEDLINE [99190893](#)  
 PUBMED [10089348](#)

REFERENCE 3 (residues 1 to 52)  
 AUTHORS Higuchi,Y. and Yasuoka,N.  
 TITLE Direct Submission  
 JOURNAL Submitted (30-SEP-1998)

COMMENT Revision History:  
 MAY 18 99 Initial Entry.

FEATURES Location/Qualifiers  
     source 1..52  
         /organism="Desulfovibrio vulgaris"  
         /db\_xref="taxon:881"  
     Region 3..51  
         /region\_name="Rubredoxin"  
         /note="rubredoxin"  
         /db\_xref="CDD:24175"  
     SecStr 3..8  
         /sec\_str\_type="sheet"  
         /note="strand 1"  
     Het join(bond(6),bond(9),bond(39),bond(42))

```
SecStr      /heterogen="( FE, 1 )"
            9..14
            /sec_str_type="sheet"
            /note="strand 2"
SecStr      16..21
            /sec_str_type="sheet"
            /note="strand 3"
SecStr      23..27
            /sec_str_type="sheet"
            /note="strand 4"
SecStr      48..51
            /sec_str_type="sheet"
            /note="strand 5"
```

ORIGIN

```
1 mkkyvctvcg yeydpaegdp dngvkpgtaf edvpadwvcp icgapksefe pa
//
```

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Books

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 2RDVA. Reports Chain A, Rubredox...[gi:5107733]

 BLink, Domains,  
Links

LOCUS 2RDV\_A 52 aa linear BCT 07-OCT-1998  
 DEFINITION Chain A, Rubredoxin From Desulfovibrio Vulgaris Miyazaki F,  
 Monoclinic Crystal Form.  
 ACCESSION 2RDV\_A  
 VERSION 2RDV\_A GI:5107733  
 DBSOURCE pdb: molecule 2RDV, chain 65, release Oct 7, 1998;  
 deposition: Oct 7, 1998;  
 class: Electron Transfer;  
 source: Mol\_id: 1; Organism\_scientific: Desulfovibrio Vulgaris;  
 Strain: Miyazaki F; Other\_details: Iam 12604;  
 Exp. method: X-Ray Diffraction.  
 KEYWORDS .  
 SOURCE Desulfovibrio vulgaris  
 ORGANISM Desulfovibrio vulgaris  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.  
 REFERENCE 1 (residues 1 to 52)  
 AUTHORS Higuchi,Y., Sugiyama,M.S., Morimoto,S., Ogata,Y., Yagi,M. and  
 Yasuoka,T.  
 TITLE Preliminary Crystallographic Study Of Two Crystal Forms Of  
 Rubredoxin From Sulfate-Reducing Bactrium  
 JOURNAL Protein Pept.Lett. 5, 175 (1998)  
 REFERENCE 2 (residues 1 to 52)  
 AUTHORS Misaki,S., Morimoto,Y., Ogata,M., Yagi,T., Higuchi,Y. and  
 Yasuoka,N.  
 TITLE Structure determination of rubredoxin from Desulfovibrio vulgaris  
 Miyazaki F in two crystal forms  
 JOURNAL Acta Crystallogr D Biol Crystallogr 55 (Pt 2), 408-413 (1999)  
 MEDLINE 99190893  
 PUBMED 10089348  
 REFERENCE 3 (residues 1 to 52)  
 AUTHORS Higuchi,Y. and Yasuoka,N.  
 TITLE Direct Submission  
 JOURNAL Submitted (07-OCT-1998)  
 COMMENT Revision History:  
 MAY 18 99 Initial Entry.  
 FEATURES Location/Qualifiers  
 source 1..52  
 /organism="Desulfovibrio vulgaris"  
 /db\_xref="taxon:881"  
 Region 3..51  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 SecStr 3..8  
 /sec\_str\_type="sheet"  
 /note="strand 1"  
 Het join(bond(6),bond(9),bond(39),bond(42))



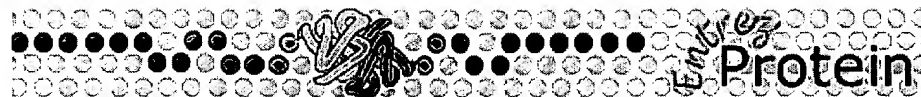
SecStr            /heterogen="( FE, 53 ) "  
                  9..14  
                  /sec\_str\_type="sheet"  
                  /note="strand 2"  
SecStr            16..21  
                  /sec\_str\_type="sheet"  
                  /note="strand 3"  
SecStr            22..27  
                  /sec\_str\_type="sheet"  
                  /note="strand 4"  
SecStr            48..52  
                  /sec\_str\_type="sheet"  
                  /note="strand 5"

ORIGIN

1 mkkyvctvcg yeydpaegdp dngvkpgtaf edvpadwvcp icgapksefe pa  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

1: 2RDVB. Reports Chain B, Rubredox...[gi:5107734]

BLink, Domains,  
Links

LOCUS 2RDV\_B 52 aa linear BCT 07-OCT-1998  
 DEFINITION Chain B, Rubredoxin From Desulfovibrio Vulgaris Miyazaki F,  
 Monoclinic Crystal Form.

ACCESSION 2RDV\_B

VERSION 2RDV\_B GI:5107734

DBSOURCE pdb: molecule 2RDV, chain 66, release Oct 7, 1998;

deposition: Oct 7, 1998;

class: Electron Transfer;

source: Mol\_id: 1; Organism\_scientific: Desulfovibrio Vulgaris;

Strain: Miyazaki F; Other\_details: Iam 12604;

Exp. method: X-Ray Diffraction.

KEYWORDS .

SOURCE Desulfovibrio vulgaris

ORGANISM Desulfovibrio vulgaris

Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1 (residues 1 to 52)

AUTHORS Higuchi,Y., Sugiyama,M.S., Morimoto,S., Ogata,Y., Yagi,M. and  
Yasuoka,T.TITLE Preliminary Crystallographic Study Of Two Crystal Forms Of  
Rubredoxin From Sulfate-Reducing Bactrium

JOURNAL Protein Pept.Lett. 5, 175 (1998)

REFERENCE 2 (residues 1 to 52)

AUTHORS Misaki,S., Morimoto,Y., Ogata,M., Yagi,T., Higuchi,Y. and  
Yasuoka,N.TITLE Structure determination of rubredoxin from Desulfovibrio vulgaris  
Miyazaki F in two crystal forms

JOURNAL Acta Crystallogr D Biol Crystallogr 55 (Pt 2), 408-413 (1999)

MEDLINE 99190893

PUBMED 10089348

REFERENCE 3 (residues 1 to 52)

AUTHORS Higuchi,Y. and Yasuoka,N.

TITLE Direct Submission

JOURNAL Submitted (07-OCT-1998)

COMMENT Revision History:

MAY 18 99 Initial Entry.

FEATURES

Location/Qualifiers

source

1..52

/organism="Desulfovibrio vulgaris"

/db\_xref="taxon:881"

Region

3..51

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

SecStr

3..8

/sec\_str\_type="sheet"

/note="strand 6"

Het

join(bond(6),bond(9),bond(39),bond(42))


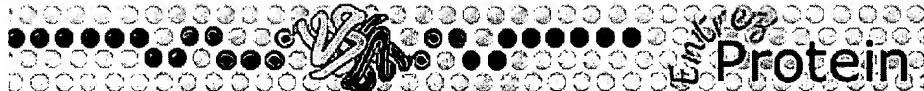
SecStr            /heterogen="( FE, 53 ) "  
                  9..14  
                  /sec\_str\_type="sheet"  
                  /note="strand 7"  
SecStr            16..19  
                  /sec\_str\_type="sheet"  
                  /note="strand 8"  
SecStr            24..27  
                  /sec\_str\_type="sheet"  
                  /note="strand 9"  
SecStr            48..51  
                  /sec\_str\_type="sheet"  
                  /note="strand 10"

ORIGIN

1 mkkyvctvcg yeydpaegdp dngvkpgtaf edvpadwvcp icgapksefe pa  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

Show:

☐ 1: [2RDVC](#). Reports Chain C, Rubredox...[gi:5107735] [BLink, Domains, Links](#)

LOCUS 2RDV\_C 52 aa linear BCT 07-OCT-1998  
 DEFINITION Chain C, Rubredoxin From *Desulfovibrio Vulgaris* Miyazaki F, Monoclinic Crystal Form.  
 ACCESSION 2RDV\_C  
 VERSION 2RDV\_C GI:5107735  
 DBSOURCE pdb: molecule 2RDV, chain 67, release Oct 7, 1998;  
 deposition: Oct 7, 1998;  
 class: Electron Transfer;  
 source: Mol\_id: 1; Organism\_scientific: *Desulfovibrio Vulgaris*;  
 Strain: Miyazaki F; Other\_details: Iam 12604;  
 Exp. method: X-Ray Diffraction.  
 KEYWORDS .  
 SOURCE *Desulfovibrio vulgaris*  
 ORGANISM *Desulfovibrio vulgaris*  
 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;  
 Desulfovibrionaceae; Desulfovibrio.  
 REFERENCE 1 (residues 1 to 52)  
 AUTHORS Higuchi,Y., Sugiyama,M.S., Morimoto,S., Ogata,Y., Yagi,M. and Yasuoka,T.  
 TITLE Preliminary Crystallographic Study Of Two Crystal Forms Of Rubredoxin From Sulfate-Reducing Bactrium  
 JOURNAL Protein Pept.Lett. 5, 175 (1998)  
 REFERENCE 2 (residues 1 to 52)  
 AUTHORS Misaki,S., Morimoto,Y., Ogata,M., Yagi,T., Higuchi,Y. and Yasuoka,N.  
 TITLE Structure determination of rubredoxin from *Desulfovibrio vulgaris* Miyazaki F in two crystal forms  
 JOURNAL Acta Crystallogr D Biol Crystallogr 55 (Pt 2), 408-413 (1999)  
 MEDLINE [99190893](#)  
 PUBMED [10089348](#)  
 REFERENCE 3 (residues 1 to 52)  
 AUTHORS Higuchi,Y. and Yasuoka,N.  
 TITLE Direct Submission  
 JOURNAL Submitted (07-OCT-1998)  
 COMMENT Revision History:  
 MAY 18 99 Initial Entry.  
 FEATURES Location/Qualifiers  
 source 1..52  
 /organism="Desulfovibrio vulgaris"  
 /db\_xref="taxon:881"  
 Region 3..51  
 /region\_name="Rubredoxin"  
 /note="rubredoxin"  
 /db\_xref="CDD:24175"  
 SecStr 3..8  
 /sec\_str\_type="sheet"  
 /note="strand 11"  
 Het join(bond(6),bond(9),bond(39),bond(42))


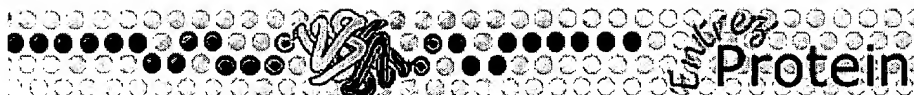
```
SecStr      /heterogen="( FE, 53 )"
9..14
/sec_str_type="sheet"
/note="strand 12"
SecStr      16..19
/sec_str_type="sheet"
/note="strand 13"
SecStr      24..27
/sec_str_type="sheet"
/note="strand 14"
SecStr      48..52
/sec_str_type="sheet"
/note="strand 15"
```

ORIGIN

```
1 mkkyvctvcg yeydpaegdp dngvkpgtaf edvpadwvcp icgapksefe pa
//
```

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

Show:

☐ 1: [1IRN](#). Reports Chain, Rubredox...[gi:1421138] [BLink, Domains, Links](#)

LOCUS 1IRN 54 aa linear BCT 13-DEC-1995  
 DEFINITION Rubredoxin (Zn-Substituted) At 1.2 Angstroms Resolution.  
 ACCESSION 1IRN  
 VERSION 1IRN GI:1421138  
 DBSOURCE pdb: molecule 1IRN, chain 32, release Dec 13, 1995;  
 deposition: Dec 13, 1995;  
 class: Electron Transport;  
 source: Mol\_id: 1; Organism\_scientific: Clostridium Pasteurianum;  
 Exp. method: X-Ray Diffraction.

KEYWORDS .  
 SOURCE Clostridium pasteurianum  
 ORGANISM [Clostridium pasteurianum](#)  
 Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 54)  
 AUTHORS Herriott,J.R., Sieker,L.C., Jensen,L.H. and Lovenberg,W.  
 TITLE Structure of rubredoxin: an x-ray study to 2.5 A resolution  
 JOURNAL J. Mol. Biol. 50 (2), 391-406 (1970)  
 MEDLINE [71027253](#)  
 PUBMED [5476919](#)

REFERENCE 2 (residues 1 to 54)  
 AUTHORS Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.  
 TITLE Refinement Of The Model Of A Protein. Rubredoxin At 1.5 Angstroms Resolution  
 JOURNAL Acta Crystallogr.,Sect.B 29, 943 (1973)

REFERENCE 3 (residues 1 to 54)  
 AUTHORS Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.  
 TITLE Crystallographic refinement of rubredoxin at 1 x 2 A degrees resolution  
 JOURNAL J. Mol. Biol. 138 (3), 615-633 (1980)  
 MEDLINE [81009589](#)  
 PUBMED [7411618](#)

REFERENCE 4 (residues 1 to 54)  
 AUTHORS Dauter,Z., Wilson,K.S., Sieker,L.C., Moulis,J.M. and Meyer,J.  
 TITLE Zinc- and iron-rubredoxins from Clostridium pasteurianum at atomic resolution: a high-precision model of a ZnS4 coordination unit in a protein  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 93 (17), 8836-8840 (1996)  
 MEDLINE [96392325](#)  
 PUBMED [8799113](#)

REFERENCE 5 (residues 1 to 54)  
 AUTHORS Dauter,Z., Wilson,K.S., Sieker,L.C., Moulis,J.M. and Meyer,J.  
 TITLE Direct Submission  
 JOURNAL Submitted (13-DEC-1995)

COMMENT Revision History:  
 JAN 5 0 Typographical  
 APR 3 96 Initial Entry.

FEATURES Location/Qualifiers

source 1..54  
/organism="Clostridium pasteurianum"  
/db\_xref="taxon:1501"  
Region 3..52  
/region\_name="Rubredoxin"  
/note="rubredoxin"  
/db\_xref="CDD:24175"  
SecStr 3..8  
/sec\_str\_type="sheet"  
/note="strand 1"  
Het join(bond(6),bond(9),bond(39),bond(42))  
/heterogen="( ZN, 1 ) Zinc Ion"  
SecStr 9..14  
/sec\_str\_type="sheet"  
/note="strand 2"  
SecStr 16..19  
/sec\_str\_type="sheet"  
/note="strand 3"  
SecStr 24..27  
/sec\_str\_type="sheet"  
/note="strand 4"  
SecStr 48..52  
/sec\_str\_type="sheet"  
/note="strand 5"

ORIGIN

1 mkkytctvcg yiynpedgdp dngvnpgtdf kdipddwvcp lcgvgkdqfe evee

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: 1IRO. Reports Chain, Rubredox...[gi:1421143]BLink, Domains,  
Links

LOCUS 1IRO 54 aa linear BCT 13-DEC-1995

DEFINITION Rubredoxin (Oxidized, Fe(III)) At 1.1 Angstroms Resolution.

ACCESSION 1IRO

VERSION 1IRO GI:1421143

DBSOURCE pdb: molecule 1IRO, chain 32, release Dec 13, 1995;  
deposition: Dec 13, 1995;  
class: Electron Transport;  
source: Mol\_id: 1; Organism\_scientific: Clostridium Pasteurianum;  
Exp. method: X-Ray Diffraction.

KEYWORDS .

SOURCE Clostridium pasteurianum

ORGANISM Clostridium pasteurianum  
Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
Clostridium.

REFERENCE 1 (residues 1 to 54)

AUTHORS Herriott,J.R., Sieker,L.C., Jensen,L.H. and Lovenberg,W.

TITLE Structure of rubredoxin: an x-ray study to 2.5 A resolution

JOURNAL J. Mol. Biol. 50 (2), 391-406 (1970)

MEDLINE 71027253

PUBMED 5476919

REFERENCE 2 (residues 1 to 54)

AUTHORS Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.

TITLE Refinement Of The Model Of A Protein. Rubredoxin At 1.5 Angstroms Resolution

JOURNAL Acta Crystallogr.,Sect.B 29, 943 (1973)

REFERENCE 3 (residues 1 to 54)

AUTHORS Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.

TITLE Crystallographic refinement of rubredoxin at 1 x 2 A degrees resolution

JOURNAL J. Mol. Biol. 138 (3), 615-633 (1980)

MEDLINE 81009589

PUBMED 7411618

REFERENCE 4 (residues 1 to 54)

AUTHORS Dauter,Z., Wilson,K.S., Sieker,L.C., Moulis,J.M. and Meyer,J.

TITLE Zinc- and iron-rubredoxins from Clostridium pasteurianum at atomic resolution: a high-precision model of a ZnS4 coordination unit in a protein

JOURNAL Proc. Natl. Acad. Sci. U.S.A. 93 (17), 8836-8840 (1996)

MEDLINE 96392325

PUBMED 8799113

REFERENCE 5 (residues 1 to 54)

AUTHORS Dauter,Z., Wilson,K.S., Sieker,L.C., Moulis,J.M. and Meyer,J.

TITLE Direct Submission

JOURNAL Submitted (13-DEC-1995)

COMMENT Revision History:  
JAN 5 0 Typographical  
APR 3 96 Initial Entry.

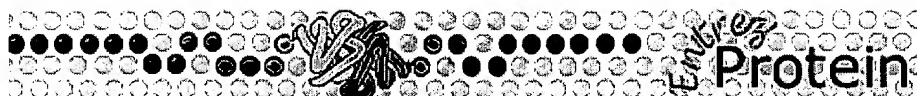
FEATURES Location/Qualifiers



```
source      1..54
            /organism="Clostridium pasteurianum"
            /db_xref="taxon:1501"
Region      3..52
            /region_name="Rubredoxin"
            /note="rubredoxin"
            /db_xref="CDD:24175"
SecStr      3..8
            /sec_str_type="sheet"
            /note="strand 1"
Het         join(bond(6),bond(9),bond(39),bond(42))
            /heterogen="( FE, 1 ) Fe(Iii) Ion"
SecStr      9..14
            /sec_str_type="sheet"
            /note="strand 2"
SecStr      16..19
            /sec_str_type="sheet"
            /note="strand 3"
SecStr      24..27
            /sec_str_type="sheet"
            /note="strand 4"
SecStr      48..52
            /sec_str_type="sheet"
            /note="strand 5"
ORIGIN
1 mkkytctvcg yiynpedgdp dngvnpgtdf kdipddwvcp lcgvgkdqfe evee
//
```

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein  for 

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default Show: 20 

Send to

File 

Get Subsequence

Feat

☐ 1: RUPE. Reports rubredoxin - Pept...[gi:65797]BLink, Domains,  
Links

LOCUS RUPE 53 aa linear BCT 24-OCT-1997  
 DEFINITION rubredoxin - Peptostreptococcus asaccharolyticus.  
 ACCESSION RUPE  
 VERSION RUPE GI:65797  
 DBSOURCE pir: locus RUPE;

summary: #length 53 #molecular-weight 5911 #checksum 6231

;

superfamily: rubredoxin; rubredoxin homology

;

PIR dates: 13-Jul-1981 #sequence\_revision 13-Jul-1981 #text\_change  
24-Oct-1997

KEYWORDS electron transfer; iron; metalloprotein.

SOURCE Peptoniphilus asaccharolyticus

ORGANISM Peptoniphilus asaccharolyticus

Bacteria; Firmicutes; Clostridia; Clostridiales;  
Peptostreptococcaceae; Peptoniphilus.

REFERENCE 1 (residues 1 to 53)

AUTHORS Bachmayer,H., Benson,A.M., Yasunobu,K.T., Garrard,W.T. and  
Whiteley,H.R.TITLE Nonheme iron proteins. IV. Structural studies of Micrococcus  
aerogenes rubredoxin

JOURNAL Biochemistry 7 (3), 986-996 (1968)

MEDLINE 68311179

PUBMED 5657864

FEATURES Location/Qualifiers

source

1..53

/organism="Peptoniphilus asaccharolyticus"

/db\_xref="taxon:1258"

Protein

1..53

/product="rubredoxin"

Region

3..51

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

Region

3..48

/region\_name="domain"

/note="rubredoxin homology #label RUB"

Site

6

/site\_type="binding"

/note="iron (Cys) "

Site

9

/site\_type="binding"

/note="iron (Cys) "

Site

38

/site\_type="binding"

/note="iron (Cys) "

Site

41

/site\_type="binding"

/note="iron (Cys)"

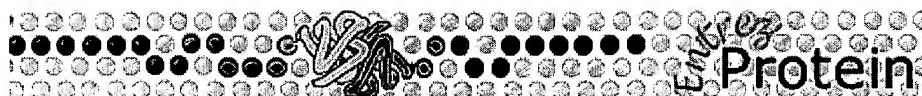
ORIGIN

1 mqkfectlcg yiydpalvgp dtpdqdgafe dvsenwvcpl cgagkedfev yed

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

1: A27537. Reports rubredoxin - Chlo...[gi:79592]

BLink, Domains,  
Links

LOCUS A27537 53 aa linear BCT 24-OCT-1997  
 DEFINITION rubredoxin - Chlorobium limicola f.sp. thiosulfatophilum.  
 ACCESSION A27537  
 VERSION A27537 GI:79592  
 DBSOURCE pir: locus A27537;

summary: #length 53 #molecular-weight 5843 #checksum 6847

;

superfamily: rubredoxin; rubredoxin homology

;

PIR dates: 05-Jun-1988 #sequence\_revision 22-Jul-1994 #text\_change  
24-Oct-1997

KEYWORDS electron transfer; iron; metalloprotein.

SOURCE Chlorobaculum thiosulfatophilum

ORGANISM Chlorobaculum thiosulfatophilum

Bacteria; Chlorobi; Chlorobia; Chlorobiales; Chlorobiaceae;  
Chlorobaculum.

REFERENCE 1 (residues 1 to 53)

AUTHORS Woolley, K.J. and Meyer, T.E.

TITLE The complete amino acid sequence of rubredoxin from the green  
phototrophic bacterium Chlorobium thiosulphatophilum strain PM

JOURNAL Eur. J. Biochem. 163 (1), 161-166 (1987)

MEDLINE 87133563

PUBMED 3816795

REMARK Chlorobium thiosulphatophilum

FEATURES Location/Qualifiers

source

1..53

/organism="Chlorobaculum thiosulfatophilum"

/db\_xref="taxon:115852"

/note="forma: thiosulfatophilum"

Protein

1..53

/product="rubredoxin"

Region

3..51

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

Region

3..49

/region\_name="domain"

/note="rubredoxin homology #label RUB"

Site

6

/site\_type="binding"

/note="iron (Cys)"

Site

9

/site\_type="binding"

/note="iron (Cys)"

Site

39

/site\_type="binding"

Site                    /note="iron (Cys) "  
                         42  
                         /site\_type="binding"  
                         /note="iron (Cys) "  
ORIGIN  
1 mqkyvcsvcg yvydpadgcp ddpidpgtgf edlpedwvcp vcgvdkdife pes  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

1: JU0127. Reports rubredoxin - "But...[gi:80482]

BLink, Domains,  
Links

LOCUS JU0127 53 aa linear BCT 24-OCT-1997  
 DEFINITION rubredoxin - 'Butyribacterium methylotrophicum'.  
 ACCESSION JU0127  
 VERSION JU0127 GI:80482  
 DBSOURCE pir: locus JU0127;

summary: #length 53 #molecular-weight 5672 #checksum 6265

;

superfamily: rubredoxin; rubredoxin homology

;

PIR dates: 31-Mar-1990 #sequence\_revision 22-Jul-1994 #text\_change  
 24-Oct-1997

KEYWORDS electron transfer; iron; metalloprotein.

SOURCE Butyribacterium methylotrophicum

ORGANISM Butyribacterium methylotrophicum

Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 53)

AUTHORS Saeki,K., Yao,Y., Wakabayashi,S., Shen,G.J., Zeikus,J.G. and  
 Matsubara,H.

TITLE Ferredoxin and rubredoxin from Butyribacterium methylotrophicum:  
 complete primary structures and construction of phylogenetic trees

JOURNAL J. Biochem. 106 (4), 656-662 (1989)

MEDLINE 90110065

PUBMED 2606914

COMMENT Rubredoxin is a nonheme iron protein and substitutes for ferredoxin  
 in some enzymatic reactions.

FEATURES Location/Qualifiers

source

1..53

/organism="Butyribacterium methylotrophicum"

/db\_xref="taxon:1487"

Protein

1..53

/product="rubredoxin"

Region

3..51

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

Region

3..49

/region\_name="domain"

/note="rubredoxin homology #label RUB"

Site

6

/site\_type="binding"

/note="iron (Cys) "

Site

9

/site\_type="binding"

/note="iron (Cys) "

Site

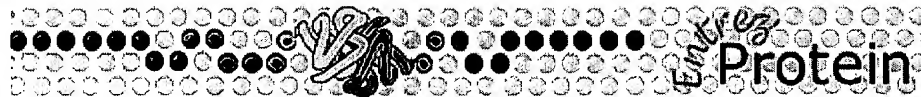
39

```

                                /site_type="binding"
                                /note="iron (Cys)"
Site      42
                                /site_type="binding"
                                /note="iron (Cys)"
ORIGIN
1 mqkyvcdicg yvydpavgdp dngvapgtaf adlpedwvcp ecgvskdefs pea
//
```

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: JU0074. Reports rubredoxin - Clos...[gi:80528]BLink, Domains,  
Links

LOCUS JU0074 54 aa linear BCT 24-OCT-1997  
 DEFINITION rubredoxin - Clostridium perfringens.  
 ACCESSION JU0074  
 VERSION JU0074 GI:80528  
 DBSOURCE pir: locus JU0074;

summary: #length 54 #molecular-weight 6004 #checksum 1502  
 ;  
 superfamily: rubredoxin; rubredoxin homology  
 ;  
 PIR dates: 28-Feb-1990 #sequence\_revision 22-Jul-1994 #text\_change  
 24-Oct-1997

KEYWORDS electron transfer; iron; metalloprotein.

SOURCE Clostridium perfringens

ORGANISM Clostridium perfringens

Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
 Clostridium.

REFERENCE 1 (residues 1 to 54)

AUTHORS Seki,Y., Seki,S., Satoh,M., Ikeda,A. and Ishimoto,M.

TITLE Rubredoxin from Clostridium perfringens: complete amino acid  
 sequence and participation in nitrate reduction

JOURNAL J. Biochem. 106 (2), 336-341 (1989)

MEDLINE 90036784

PUBMED 2553684

COMMENT The protein is reduced with NADH in the presence of a specific  
 NAD(P)H oxidoreductase.

FEATURES Location/Qualifiers

source

1..54

/organism="Clostridium perfringens"

/db\_xref="taxon:1502"

Protein

1..54

/product="rubredoxin"

Region

3..49

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

Region

3..49

/region\_name="domain"

/note="rubredoxin homology #label RUB"

Site

6

/site\_type="binding"

/note="iron (Cys) "

Site

9

/site\_type="binding"

/note="iron (Cys) "

Site

39

/site\_type="binding"



Site                    /note="iron (Cys) "  
                         42  
                         /site\_type="binding"  
                         /note="iron (Cys) "  
ORIGIN  
//        1 mkkficdvvg yiydpavgdp dngvepgtef kdipddwvcp lcgvdksqfs etee

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

1: A33182. Reports rubredoxin - Clos...[gi:98633]

BLink, Domains,  
Links

LOCUS A33182 53 aa linear BCT 24-OCT-1997  
 DEFINITION rubredoxin - Clostridium sticklandii.  
 ACCESSION A33182  
 VERSION A33182 GI:98633  
 DBSOURCE pir: locus A33182;

summary: #length 53 #molecular-weight 5889 #checksum 6706

;

superfamily: rubredoxin; rubredoxin homology

;

PIR dates: 03-Feb-1994 #sequence\_revision 03-Feb-1994 #text\_change  
24-Oct-1997

KEYWORDS electron transfer; iron; metalloprotein.

SOURCE Clostridium sticklandii

ORGANISM Clostridium sticklandii

Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;  
Clostridium.

REFERENCE 1 (residues 1 to 53)

AUTHORS Meyer, J., Gagnon, J. and Moulis, J.M.

TITLE Direct Submission

JOURNAL Submitted (??-APR-1991) to the Protein Sequence Database

FEATURES Location/Qualifiers

source

1..53

/organism="Clostridium sticklandii"

/db\_xref="taxon:1511"

Protein

1..53

/product="rubredoxin"

Site

1

/site\_type="modified"

/note="N-formylmethionine"

Region

3..51

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

Region

3..49

/region\_name="domain"

/note="rubredoxin homology #label RUB"

Site

6

/site\_type="binding"

/note="iron (Cys) "

Site

9

/site\_type="binding"

/note="iron (Cys) "

Site

39

/site\_type="binding"

/note="iron (Cys) "

Site

42

/site\_type="binding"  
/note="iron (Cys)"

ORIGIN

1 mtkyvctvcg yvydpevgdp dnninpgtsf qdipedwvcp lcgvgkdqfe eea  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: JX0241. Reports rubredoxin - Desu...[gi:320090]

 BLink, Domains,  
Links

LOCUS JX0241 52 aa linear BCT 24-OCT-1997  
 DEFINITION rubredoxin - Desulfovibrio vulgaris (strain Miyazaki).  
 ACCESSION JX0241  
 VERSION JX0241 GI:320090  
 DBSOURCE pir: locus JX0241;

summary: #length 52 #molecular-weight 5598 #checksum 1997

;

superfamily: rubredoxin; rubredoxin homology

;

PIR dates: 10-Jun-1993 #sequence\_revision 22-Jul-1994 #text\_change 24-Oct-1997

KEYWORDS electron transfer; iron; metalloprotein.

SOURCE Desulfovibrio vulgaris

ORGANISM Desulfovibrio vulgaris

Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio.

REFERENCE 1 (residues 1 to 52)

AUTHORS Shimizu,F., Ogata,M., Yagi,T., Wakabayashi,S. and Matsubara,H.

TITLE Amino acid sequence and function of rubredoxin from Desulfovibrio vulgaris Miyazaki

JOURNAL Biochimie 71 (11-12), 1171-1177 (1989)

MEDLINE 90234754

PUBMED 2561345

FEATURES

Location/Qualifiers

source

1..52

/organism="Desulfovibrio vulgaris"

/db\_xref="taxon:881"

Protein

1..52

/product="rubredoxin"

Site

1

/site\_type="modified"

/note="N-formylmethionine (partial)"

Region

3..51

/region\_name="Rubredoxin"

/note="rubredoxin"

/db\_xref="CDD:24175"

Region

3..49

/region\_name="domain"

/note="rubredoxin homology #label RUB"

Site

6

/site\_type="binding"

/note="iron (Cys)"

Site

9

/site\_type="binding"

/note="iron (Cys)"

Site

39

Site

/site\_type="binding"  
/note="iron (Cys) "  
42  
/site\_type="binding"  
/note="iron (Cys) "

ORIGIN

1 mkkyvctvcg yeydpaegdp dngvkpgtaf edvpadwvcp icgapksefe pa

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



IAEGAMSEGADVVRVYYLHEDDRSEIVKDI LD SHAIALGAPAIYDEPYPSVGDLLMYLR  
GLKFNRTGQRRAMVFGSMGGRGGATGTMQKLLADAGFDVMEADEIYYVPNNEELDACF  
EAGRRLAGDLNE"

gene 1542..1922  
/gene="orfX"

CDS 1542..1922  
/gene="orfX"  
/note="contains one of two motifs responsible for ligating  
two Fe atoms in non-heme, non-sulfur di-iron containing  
proteins"  
/codon\_start=1  
/transl\_table=11  
/product="unknown"  
/protein\_id="AAB53660.1"  
/db\_xref="GI:710357"  
/translation="MNENITGVCRGTELENVVDANFRGECSEVGIYLMARQAQREGL  
PEVAEALRRIALEEAHAARFAELNGVIRENLRENLEMMLEGEAAANREKREAALRAR  
DAVLMRPMTSLMRAQGTREGMLPC"

gene 1957..2490  
/gene="rdxA"

CDS 1957..2490  
/gene="rdxA"  
/note="contains a N-terminal located rubredoxin domain"  
/codon\_start=1  
/transl\_table=11  
/product="unknown"  
/protein\_id="AAB53661.1"  
/db\_xref="GI:710358"  
/translation="MKICRICGYQIPEGEFNLLLEDGWVCPRCGVGKEELQDSAEPLGG  
RDPLMLIFRAMTVGLWRVLGNGSQGV TREMGSVIADNIRHGDDPLKSAADYFIEHGFA  
ASISTDTENFALNVKNCSFYGFCCSLEEDGVLLSTCPYANTAAVLETTGYRYRIKR  
NKGDHGHIIIEFSRISKK"

## ORIGIN

```
1 ggatccggca tttcaactat gaggcccgc ttcctgaggt ctgagaggtg ttttgatact
61 gtggacttgg atttatctgt gattctgact atttcctgga gctgagtgt ccctttttaa
121 gttcggagag tatgagcgac ttgacagtgc tttctattca atcgattcca gtttcggttg
181 agaatatcgt gaaattatgt ctccctcttca ttggcccatg ttatgtctga aggactatat
241 tactgtttca ataccatgga acagaaaaaa ttatatagaa gttcgtatag tatagtacat
301 tcgatgatat acgaactaaa tgtgtggtga tataatgaag gccagagcag agaagatagc
361 agatggatta tactggactg gtgtccttga ctgggacatc agaaactacc acggatacac
421 acttcagggg acaacctaca acgcctacct tgtattcggga gatgaaggtg tggcgctgat
481 tgacaactca tatccaggga ccttcaggga gctcatggca aggatggagg atgcattcaa
541 ccgtgaggga agagagatga gagtggactt catagtccag aaccatgttg aaagggacca
601 cagcggagtg ctggtggaac ttcagaggag gtccctgag gcagagatcc atgcacaga
661 agttgccgtg gagggactgc tcaagcatta cccggccctt gagggcacag agttccgcac
721 agtgaagacc ggggattcca tcgacctcgg aggaaggacc ctcaccttcc ttgaggcacc
781 cctgctccac tggccagaca gcatgttcac cttccttgat accggcatac tgttctcaa
841 cgacgccttt ggacagcacc tctgctatcc acagcgctc gacaccgaaa taccgaata
901 catactcatg gatgctgcca aaaaattcta tgcaaacctc ataacacccc tatcaaagct
961 tgtgctcagg aagttcgatg aggtgaagga actcggcctc cttgataaga tagggatgat
1021 agcgcctcc catggccaga tatggaccga acccatgaag ataatagaag catacgccgc
1081 ctgggccaca ggtaaggtga agaagaaggt caccgtaatc tatgatacga tgcaccactc
1141 cacagccatg atggcccatg caatagcaga gggggccatg agcgaggggg ctgatgtgag
1201 ggtctactac ctccatgagg acgaccgcag cgagatcgtg aaggacatac ttgacagtca
1261 cgccattgcc ctcgagcac ccgcaatcta cgatgaacct taccgagcg ttggagacct
1321 cctgatgtac ctccgtggtc tgaagttcaa caggaccggg cagaggaggg ccatggtatt
1381 cgggtcaatg ggtggcaggg gaggtgccac cgggaccatg cagaaactcc ttgcggtatg
1441 tggattcgat gtcattggag cggtatgaaat ctactacgtc ccgaacaatg aggaactcga
1501 tgcattgctt gaggtggaa gaaggcttgc aggtgatctc aatgaatgaa aacatcaccg
1561 gtgtctgcag gggcaccgaa cttgagaatg ttgtggatgc aaacttcagg ggagagtga
```

1621 gtgaggtcgg tatctacctt gcaatggcaa gacaggccca gcgtgagggc ctccccgagg  
1681 ttgcagaggc cttagaaga atagcccttg aggaagctgc ccatgcagcc cgctttgcag  
1741 agctaaacgg tgtgatcagg gaaaacctca gggaaaacct tgagatgatg cttgaggggg  
1801 aggaggcggc taacaggagg aaacgtgagg cagccctcag ggcaagggat gcggtgttga  
1861 tgaggcccat gacttctttg atgagagctc aaggggacgag ggaaggcatg ctgccatgct  
1921 gaggggactc ctccagaggt acttcagggt ataaccatga agatctgcag aatatgcggc  
1981 tatcagatcc cagaaggtga attcaacctg ctggaggatg gctgggtgtg tccccgggtg  
2041 ggtgttgagg aggaggaact ccaggattcc gccgagcccc tgggggggtcg ggacccccctc  
2101 atgcttattt tcagggccat gactgtgggg ctctggaggg ttctgggtaa tggatcccag  
2161 ggagttacaa gggagatggg gtctgtgatt gcagacaaca tcaggcacgg ggatgatccc  
2221 ctgaaatcag ccgcagacta cttcatcgaa catggatttg cagcgtccat cagcacagac  
2281 actgaaaact ttgccctcaa tgttaaaaac tgcagtttct atggattctg ttgttctactg  
2341 gaggaggatg ggggtgctttt atcaacctgc ccctatgcaa acacggccgc tgcagtcctt  
2401 gaaaggacca cgggatacag gtaccgtata aaacgaaata agggggacca tggacacatc  
2461 attgaatttt cacggatatc aaaaaaatag gtaggggttc gagattaact tcacaacggg  
2521 gccaccgaag cccctctgat tcagaaatca gcatagggtc tctccatacc cgcacgcttc  
2581 agtatatcct ccgcatgttt gagggaccgg tcgctgccat caaccaggag gtggacggct  
2641 ccctcagccc cctggacgcc tccagctgca acaggctctg catccacacc cgccagaagc  
2701 ctgagggcgt ccacctctgt gaagacctcg ccgggtacag gtaacattcc gggaccctca  
2761 gtgccggctg aattcattcg gagcgtgta ctgtaaaggc cgcttggtat catcttttca  
2821 agtccaaccg ggactatgag ctc

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10





PubMed

BLAST

Structure

Taxonomy

OMIM

Help?

Cn3d

**Description:** Alzheimer's Disease Amyloid Beta-Peptide (Residues 1 - 28) (E.C. Number Not Assigned) (Nmr, Minimized Average Structure).

**Deposition:** J.Talafous, K.J.Marcinowski, G.Klopman & M.G.Zagorski, 21-Oct-94

**Taxonomy:** Homo sapiens

**Reference:** PubMed **MMDB:** 230 **PDB:** 1AMB

View 3D Structure

of

Best Model

with

Cn3D

Display

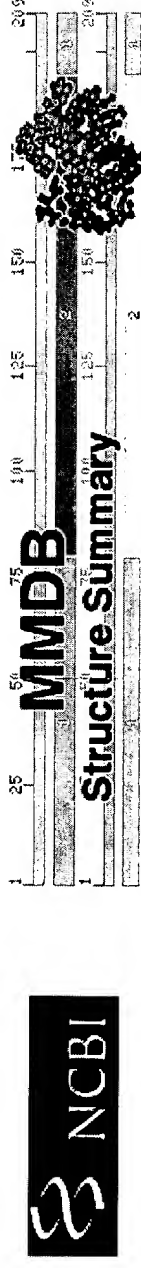
NEW

Get Cn3D 4.1!



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", **Nucleic Acids Res.** **2003 Jan**; **31**(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

**Description:** Alzheimer's Disease Amyloid Beta-Peptide (Residues 1 - 28) (E.C. Number Not Assigned) (Nmr, 5 Structures).

**Deposition:** J.Talafous, K.J.Marcinowski, G.Klopman & M.G.Zagorski, 14-Nov-94

**Taxonomy:** Homo sapiens

**Reference:** PubMed **MMDB: 231** **PDB: 1AMC**

of  with    [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", *Nucleic Acids Res.* 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLN](#) | [NIH](#)





PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

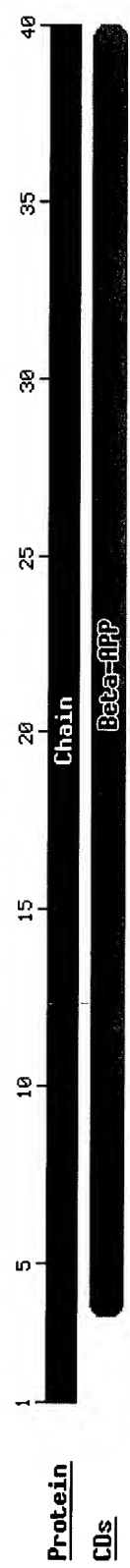
**Description:** The Solution Structure Of Amyloid Beta-Peptide (1-40) In A Water-Micelle Environment. Is The Membrane-Spanning Domain Where We Think It Is? Nmr, 10 Structures.

**Deposition:** M.Coles, W.Bicknell, A.A.Watson, D.P.Fairlie & D.J.Craik, 7-Apr-98

**Taxonomy:** Homo sapiens

**Reference:** PubMed **MMDB:** 7992 **PDB:** 1BA4

of  with    [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", **Nucleic Acids Res.** 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLN](#) | [NIH](#)



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

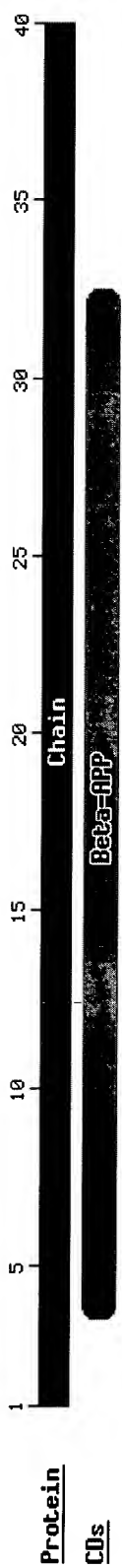
**Description:** Solution Structure Of The Methionine-Oxidized Amyloid Beta-Peptide (1-40). Does Oxidation Affect Conformational Switching? Nmr, 10 Structures.

**Deposition:** A.A.Watson, D.P.Fairlie & D.J.Craik, 22-Apr-98

**Taxonomy:** [Homo sapiens](#)

**Reference:** [PubMed](#) **MMDB:** [7993](#) **PDB:** [1BA6](#)

[View 3D Structure](#) of [Best Model](#) with [Cn3D](#) [Display](#) [NEW](#) [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DJ, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", **Nucleic Acids Res.** 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)





**Description:** Solution Nmr Structure Of Amyloid Beta[e16], Residues 1-28, 14 Structures.

**Deposition:** S.-A.Poulsen, A.A.Watson & D.J.Craik, 23-Jun-98

**Taxonomy:** Homo sapiens

**Reference:** PubMed **MMDB:** 8655 **PDB:** 1BJB

of  with    [Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: Entrez's 3D-structure database", **Nucleic Acids Res.** 2003 Jan; 31(1): 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLN](#) | [NIH](#)



NCBI

MMDB

Structure Summary



PubMed BLAST Structure Taxonomy OMIM Help? Cn3d

Description: Solution Nmr Structure Of Amyloid Beta[f16], Residues 1-28, 15 Structures.

Deposition: S.-A.Poulsen, A.A.Watson & D.J.Craik, 23-Jun-98

Taxonomy: Homo sapiens

Reference: PubMed **MMDB: 9179** **PDB: 1BJC**

View 3D Structure

 of 

Best Model

 with 

Cn3D

Display


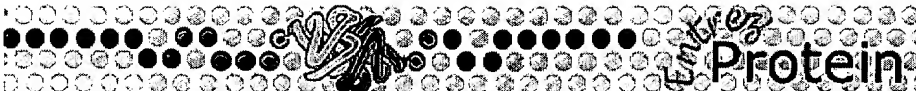
NEW

[Get Cn3D 4.1!](#)



**Citing MMDB:** Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "MMDB: *Entrez's 3D-structure database*", **Nucleic Acids Res.** **2003 Jan**; **31(1)**: 474-7.

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Boo](#)

Search  for

☐ 1: [AAA37139](#). Reports beta-amyloid prot...[gi:309085] BLink, Domains, Links

LOCUS AAA37139 695 aa linear ROD 11-JUN-1993  
 DEFINITION beta-amyloid protein.  
 ACCESSION AAA37139  
 VERSION AAA37139.1 GI:309085  
 DBSOURCE locus MUSABPPA accession [M18373.1](#)  
 KEYWORDS .  
 SOURCE Mus musculus (house mouse)  
 ORGANISM Mus musculus  
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;  
 Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.  
 REFERENCE 1 (residues 1 to 695)  
 AUTHORS Yamada,T., Sasaki,H., Furuya,H., Miyata,T., Goto,I. and Sakaki,Y.  
 TITLE Complementary DNA for the mouse homolog of the human amyloid beta  
 protein precursor  
 JOURNAL Biochem. Biophys. Res. Commun. 149 (2), 665-671 (1987)  
 MEDLINE [88106489](#)  
 PUBMED [3322280](#)  
 COMMENT On Jul 26, 1993 this sequence version replaced gi:191569.  
 Draft entry and computer readable copy of sequence [1] kindly  
 provided by T.Yamada (17-MAR-1988).  
 Method: conceptual translation.  
 FEATURES Location/Qualifiers  
 source 1..695  
 /organism="Mus musculus"  
 /db\_xref="taxon:10090"  
Protein 1..695  
 /name="beta-amyloid protein"  
Region 24..188  
 /region\_name="amyloid A4"  
 /note="A4\_EXTRA"  
 /db\_xref="CDD:22659"  
Region <309..505  
 /region\_name="ATPase involved in DNA repair [DNA  
 replication, recombination, and repair]"  
 /note="SbcC"  
 /db\_xref="CDD:10293"  
Region 600..>630  
 /region\_name="Beta-amyloid peptide (beta-APP)"  
 /note="Beta-APP"  
 /db\_xref="CDD:5537"  
CDS 1..695  
 /coded\_by="M18373.1:139..2226"

## ORIGIN

```

1 mlpslallll aawtvrale v ptdgnaglla epqiamfcgk lnmhmnvqng kwesdpsgtk
61 tcigtkegil gycqevypel qitnvveanq pvtiqnwckr grkqckthth ivipyrclvg
121 efvsdallvp dkckflhqer mdvcethlhw htvaketcse kstnlhdygm llpcgidkfr
181 gvefvccpla eesdsvsdsad aeedsdsvvw vgadtdyadg gedkvvevae eeevadveee
241 eadddedved gdeveeeae pyeater tt stattttttt esveevrvp ttaastpdav

```



301 dkyletpgde nehahfqkak erleakhrer msqvmrewee aerqaknlpk adkkaviqhf  
361 gekvesleqe aanerqqqlve thmarveaml ndrrrldlen yiialqavpp rphhvfnmlk  
421 kyvraeqkdr qhtlkhfehv rmvdpkkatq irsqvmthlr viyermnqsl sllynvpava  
481 eeiqdevdel lqkeqnysdd vlanmisepr isygndalmp sltetkttve llpvngefsl  
541 ddlqpwhpfg vdsvpanten evepvdarpa adrglttrpg sgltniktee isevkmdaef  
601 ghdsgfevrh qklvffaedv gsnkgaiigl mvggvviatv ivitlvmlkk kqytsihhgv  
661 vevdaavtpe erhlsmqqn gyenptykff eqmqn

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



[Entrez](#) [PubMed](#) [Nucleotide](#) [Protein](#) [Genome](#) [Structure](#) [PMC](#) [Taxonomy](#) [Boo](#)

Search  for

Limits  default   File

☐ 1: [AAA51726](#). Reports beta-amyloid A4...[gi:178573] [BLink, Domains, Links](#)

LOCUS AAA51726 264 aa linear PRI 31-OCT-1994  
 DEFINITION beta-amyloid A4.  
 ACCESSION AAA51726  
 VERSION AAA51726.1 GI:178573  
 DBSOURCE locus HUMAMY4A accession [M18734.1](#)  
 KEYWORDS .  
 SOURCE Homo sapiens (human)  
 ORGANISM [Homo sapiens](#)  
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;  
 Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (residues 1 to 264)  
 AUTHORS Zain,S.B., Salim,M., Chou,W.G., Sajdel-Sulkowska,E.M., Majocha,R.E.  
 and Marotta,C.A.  
 TITLE Molecular cloning of amyloid cDNA derived from mRNA of the  
 Alzheimer disease brain: coding and noncoding regions of the fetal  
 precursor mRNA are expressed in the cortex  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 85 (3), 929-933 (1988)  
 MEDLINE [88124954](#)  
 PUBMED [2893379](#)  
 COMMENT Method: conceptual translation.  
 FEATURES Location/Qualifiers  
 source 1..264  
 /organism="Homo sapiens"  
 /db\_xref="taxon:9606"  
 /map="21q21.2"  
[Protein](#) 1..264  
 /name="beta-amyloid A4"  
[Region](#) 169..>199  
 /region\_name="Beta-amyloid peptide (beta-APP)"  
 /note="Beta-APP"  
 /db\_xref="CDD:5537"  
[CDS](#) 1..264  
 /gene="APP"  
 /coded\_by="M18734.1:<1..797"  
 /codon\_start=2  
 /db\_xref="GDB:G00-119-692"

## ORIGIN

```


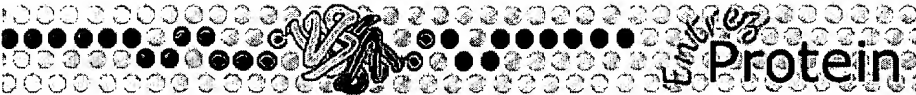
1 htlkhfehvr mvdpkkaaqi rsqvmthlr v iyer mnqsls llynvpavae eiqdevdell
61 qkeqnysddv lanmisepri sygndalmps ltetkttvel lpvngefsld dlqpwhsfga
121 dsvpantene vepvdarpaa drgltrrpgs gltnikteei sevkmdaefr hdsgeyvhq
181 klvffaedvg snkgaiiglm vggvviatvi vitlvmlkkk qytsihhgvv evdaavtpee
241 rhlskmqngn yenptykffe qmqn

```

//

Nov 8 2004 13:44:10

**THIS PAGE BLANK (USPTO)**

[Entrez](#)
[PubMed](#)
[Nucleotide](#)
[Protein](#)
[Genome](#)
[Structure](#)
[PMC](#)
[Taxonomy](#)
[Boo](#)

Search  for

☐ 1: [AAA71550](#). Reports Sequence 1 from p...[gi:912096] [BLink](#), [Links](#)

LOCUS AAA71550 40 aa linear PAT 26-JUL-1995  
 DEFINITION Sequence 1 from patent US 5434050.  
 ACCESSION AAA71550  
 VERSION AAA71550.1 GI:912096  
 DBSOURCE accession [AAA71550.1](#)  
 KEYWORDS .  
 SOURCE Unknown.  
 ORGANISM Unknown.  
 Unclassified.  
 REFERENCE 1 (residues 1 to 40)  
 AUTHORS Maggio, J.E. and Mantyh, P.W.  
 TITLE Labelled .beta.-amyloid peptide and methods of screening for  
 Alzheimer's disease  
 JOURNAL Patent: US 5434050-A 1 18-JUL-1995;  
 FEATURES Location/Qualifiers  
 source 1..40  
 /organism="unknown"  
 Region 4..40  
 /region\_name="Beta-amyloid peptide (beta-APP)"  
 /note="Beta-APP"  
 /db\_xref="CDD:5537"  
 ORIGIN  
 1 daefrhdsgy evhhqklvff aedvgsnkg a iiglmvggvv  
 //

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein  for 

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default 

Show:

20 

Send to

File 

Get Subsequence

Feat

☐ 1: AAA71551. Reports Sequence 2 from p...[gi:912097]

BLink, Links

LOCUS AAA71551 42 aa linear PAT 26-JUL-1995  
 DEFINITION Sequence 2 from patent US 5434050.  
 ACCESSION AAA71551  
 VERSION AAA71551.1 GI:912097  
 DBSOURCE accession [AAA71551.1](#)  
 KEYWORDS .  
 SOURCE Unknown.  
 ORGANISM Unknown.  
 Unclassified.  
 REFERENCE 1 (residues 1 to 42)  
 AUTHORS Maggio, J.E. and Mantyh, P.W.  
 TITLE Labelled .beta.-amyloid peptide and methods of screening for  
 Alzheimer's disease  
 JOURNAL Patent: US 5434050-A 2 18-JUL-1995;  
 FEATURES Location/Qualifiers  
 source 1..42  
 /organism="unknown"  
 Region 4..42  
 /region\_name="Beta-amyloid peptide (beta-APP)"  
 /note="Beta-APP"  
 /db\_xref="CDD:5537"  
 ORIGIN  
 1 daefrhdsgy evhhqklvff aedvgsnkg a iiglmvggv ia  
 //

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat


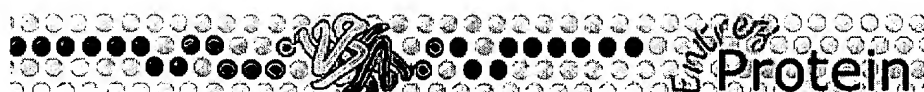

☐ 1: AAA75654. Reports Sequence 1 from p...[gi:997353]

BLink, Links

LOCUS AAA75654 16 aa linear PAT 26-SEP-1995  
DEFINITION Sequence 1 from patent US 5441870.  
ACCESSION AAA75654  
VERSION AAA75654.1 GI:997353  
DBSOURCE accession [AAA75654.1](#)  
KEYWORDS .  
SOURCE Unknown.  
ORGANISM Unknown.  
Unclassified.  
REFERENCE 1 (residues 1 to 16)  
AUTHORS Seubert,P.A., Schenk,D.B. and Fritz,L.C.  
TITLE Methods for monitoring cellular processing of .beta.-amyloid  
precursor protein  
JOURNAL Patent: US 5441870-A 1 15-AUG-1995;  
FEATURES Location/Qualifiers  
source 1..16  
/organism="unknown"  
ORIGIN  
1 gsgltnikte eisevk  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Boo

Search Protein  for

Limits Preview/Index History Clipboard Details

default   File

☐ 1: AAB13827. Reports Sequence 8 from p...[gi:1610848] BLink, Links

LOCUS AAB13827 770 aa linear PAT 07-OCT-1996

DEFINITION Sequence 8 from patent US 5525714.

ACCESSION AAB13827

VERSION AAB13827.1 GI:1610848

DBSOURCE accession AAB13827.1

KEYWORDS .

SOURCE Unknown.

ORGANISM Unknown.

Unclassified.

REFERENCE 1 (residues 1 to 770)

AUTHORS Van Broeckhoven,C., Martin,J.-J., Hendriks,L. and Cras,P.

TITLE Mutated form of the .beta.-amyloid precursor protein gene

JOURNAL Patent: US 5525714-A 8 11-JUN-1996;

FEATURES

Location/Qualifiers

source 1..770

/organism="unknown"

Region 24..188

/region\_name="amyloid A4"

/note="A4\_EXTRA"

/db\_xref="CDD:22659"

Region 291..342

/region\_name="BPTI/Kunitz family of serine protease inhibitors"

/note="KU"

/db\_xref="CDD:5332"

Region <384..580

/region\_name="ATPase involved in DNA repair [DNA replication, recombination, and repair]"

/note="SbcC"

/db\_xref="CDD:10293"

Region 675..>705

/region\_name="Beta-amyloid peptide (beta-APP)"

/note="Beta-APP"

/db\_xref="CDD:5537"

ORIGIN

```
1 mlpglallll aawtaralev ptdgnaglla epqiamfcgr lnmhmnvqng kwdsdpsgtk
61 tcidtkegil gycqevypel qitnvveanq pvtiqnwckr grkqckthph fvipyrclyg
121 efvsdallvp dkckflhqer mdvcethlhw htvaketcse kstnlhdygm llpcgidkfr
181 gvefvccpla eesdnvdsad aeeddsdvww ggadtdyadg sedkvvevae eeveaeveee
241 eadddedded gdeveeeaae pyeeatertt siattttttt esveevvrev csegaetgpc
301 ramisrwyfd vtegkcapff yggcggnrnn fdteeycmav cgsamsqsl1 kttqeplard
361 pvklpttaas tpdavdkyle tpgdenegah fqkakerlea khrermsqvm reweeaerqa
421 knlpkadkka viqhfgekve sleqeaaner qqlvethmar veamlndrrr lalenyital
481 qavpprprhv fnmlkkyvra eqkdrqhtlk hfehvrmdvp kkaaqrsvq mthlrviyer
541 mnqslsllyn vpavaeeiqd evdellqkeq nysddvlanm isepriygn dalmpsltet
601 kttvellpvn gefslddlqp whsfgadsvp antenevepv darpaadrgl ttrpgsgltn
661 ikteeisevk mdaeifrhdsg yevvhqklvf faedvgsnkg aiiglmvggv viatvivitl
721 vmlkkkkqyts ihhgvvevda avtpeerhls kmqqngyenp tykffeqmqn
```

//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: AAB15507. Reports Sequence 1 from p...[gi:1612527]

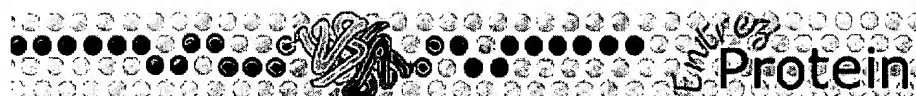
BLink, Links

LOCUS AAB15507 43 aa linear PAT 07-OCT-1996  
DEFINITION Sequence 1 from patent US 5552426.  
ACCESSION AAB15507  
VERSION AAB15507.1 GI:1612527  
DBSOURCE accession AAB15507.1  
KEYWORDS .  
SOURCE Unknown.  
ORGANISM Unknown.  
Unclassified.  
REFERENCE 1 (residues 1 to 43)  
AUTHORS Lunn,W.H.W., Monn,J.A. and Zimmerman,D.M.  
TITLE Methods for treating a physiological disorder associated with  
.beta.-amyloid peptide  
JOURNAL Patent: US 5552426-A 1 03-SEP-1996;  
FEATURES Location/Qualifiers  
source 1..43  
/organism="unknown"  
ORIGIN  
1 daefrhdsy evhhqklvff aedvgsnkga iiglmvggvv iat  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10





Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

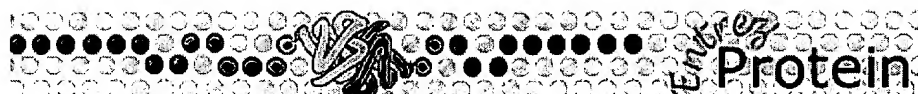
1: AAB15508. Reports Sequence 2 from p...[gi:1612528]

BLink, Links

LOCUS AAB15508 40 aa linear PAT 07-OCT-1996  
 DEFINITION Sequence 2 from patent US 5552426.  
 ACCESSION AAB15508  
 VERSION AAB15508.1 GI:1612528  
 DBSOURCE accession AAB15508.1  
 KEYWORDS .  
 SOURCE Unknown.  
 ORGANISM Unknown.  
 Unclassified.  
 REFERENCE 1 (residues 1 to 40)  
 AUTHORS Lunn,W.H.W., Monn,J.A. and Zimmerman,D.M.  
 TITLE Methods for treating a physiological disorder associated with  
 .beta.-amyloid peptide  
 JOURNAL Patent: US 5552426-A 2 03-SEP-1996;  
 FEATURES Location/Qualifiers  
 source 1..40  
 /organism="unknown"  
 Region 4..40  
 /region\_name="Beta-amyloid peptide (beta-APP)"  
 /note="Beta-APP"  
 /db\_xref="CDD:5537"  
 ORIGIN  
 1 daefrhdsy evhhqklvff aedvgsnkgg iiglmvggvv  
 //

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

1: AAB19991. Reports amyloid precursor...[gi:236721]

BLink, Domains,  
Links

LOCUS AAB19991 49 aa linear PRI 07-MAY-1993

DEFINITION amyloid precursor protein; APP [Homo sapiens].

ACCESSION AAB19991

VERSION AAB19991.1 GI:236721

DBSOURCE locus S57665 accession S57665.1

KEYWORDS .

SOURCE Homo sapiens (human)

ORGANISM Homo sapiens

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;  
Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (residues 1 to 49)

AUTHORS Murrell,J., Farlow,M., Ghetti,B. and Benson,M.D.

TITLE A mutation in the amyloid precursor protein associated with  
hereditary Alzheimer's disease

JOURNAL Science 254 (5028), 97-99 (1991)

MEDLINE 92022553

PUBMED 1925564

REMARK GenBank staff at the National Library of Medicine created this  
entry [NCBI gibbsq 57667] from the original journal article.

COMMENT Method: conceptual translation.

FEATURES

Location/Qualifiers

source

1..49

/organism="Homo sapiens"

/db\_xref="taxon:9606"

Protein

1..49

/product="amyloid precursor protein"

/note="transmembrane domain; APP"

Region

&lt;1..25

/region\_name="Beta-amyloid peptide (beta-APP)"

/note="Beta-APP"

/db\_xref="CDD:5537"

CDS

1..49

/gene="amyloid precursor protein, APP"

/coded\_by="S57665.1:109..255"

ORIGIN

1 vffaedvgsn kgaiiglmvg gvviatvifi tlvmlkkkqy tsihhgvve

//

Disclaimer | Write to the Help Desk  
NCBI | NLM | NIH

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Book

Search Protein for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

1: AAB23544. Reports beta-amyloid pept...[gi:257056]

BLink, Domains,  
Links

LOCUS AAB23544 33 aa linear PRI 08-MAY-1993

DEFINITION beta-amyloid peptide, A beta {N-terminal} [human, cerebrospinal fluid, conditioned medium of mixed-brain cell cultures, Peptide Partial, 33 aa].

ACCESSION AAB23544

VERSION AAB23544.1 GI:257056

DBSOURCE accession AAB23544.1

KEYWORDS .

SOURCE Homo sapiens (human)

ORGANISM Homo sapiens

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (residues 1 to 33)

AUTHORS Seubert,P., Vigo-Pelfrey,C., Esch,F., Lee,M., Dovey,H., Davis,D., Sinha,S., Schlossmacher,M., Whaley,J., Swindlehurst,C. et,al.

TITLE Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids

JOURNAL Nature 359 (6393), 325-327 (1992)

MEDLINE 93024877

PUBMED 1406936

REMARK GenBank staff at the National Library of Medicine created this entry [NCBI gibbsq 114715] from the original journal article.

COMMENT Method: sequenced peptide, ordered by homology.

FEATURES Location/Qualifiers

source

1..33

/organism="Homo sapiens"

/db\_xref="taxon:9606"

Protein

1..33

/product="beta-amyloid peptide"

/note="A beta"

Region

4..&gt;33

/region\_name="Beta-amyloid peptide (beta-APP)"

/note="Beta-APP"

/db\_xref="CDD:5537"

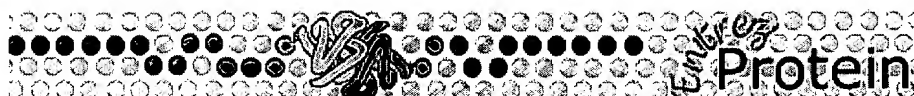
ORIGIN

1 daefrhds gy evhhqklvff aedvgsnkga iig

//

Disclaimer | Write to the Help Desk  
NCBI | NLM | NIH

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat


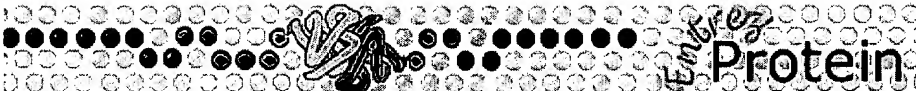
☐ 1: [AAB28602](#). Reports beta-amyloid pept...[gi:435822]

 BLink, Domains,  
Links

LOCUS AAB28602 28 aa linear PRI 18-DEC-1993  
 DEFINITION beta-amyloid peptide, A beta {N-terminal} [human, patient with Alzheimer's disease, cerebrospinal fluid, Peptide Partial, 28 aa].  
 ACCESSION AAB28602  
 VERSION AAB28602.1 GI:435822  
 DBSOURCE accession [AAB28602.1](#)  
 KEYWORDS .  
 SOURCE Homo sapiens (human)  
 ORGANISM [Homo sapiens](#)  
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (residues 1 to 28)  
 AUTHORS Vigo-Pelfrey,C., Lee,D., Keim,P., Lieberburg,I. and Schenk,D.B.  
 TITLE Characterization of beta-amyloid peptide from human cerebrospinal fluid  
 JOURNAL J. Neurochem. 61 (5), 1965-1968 (1993)  
 MEDLINE [94045685](#)  
 PUBMED [8229004](#)  
 REMARK GenBank staff at the National Library of Medicine created this entry [NCBI gibbsq 139006] from the original journal article.  
 COMMENT Method: direct peptide sequencing.  
 FEATURES Location/Qualifiers  
     source 1..28  
         /organism="Homo sapiens"  
         /db\_xref="taxon:9606"  
     Protein 1..28  
         /product="beta-amyloid peptide"  
         /note="A beta"  
     Region 4..>27  
         /region\_name="Beta-amyloid peptide (beta-APP)"  
         /note="Beta-APP"  
         /db\_xref="CDD:[5537](#)"  
 ORIGIN  
   1 daefrhds gy evhhqklvff aedvgsnx  
 //

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

[Entrez](#)   [PubMed](#)   [Nucleotide](#)   [Protein](#)   [Genome](#)   [Structure](#)   [PMC](#)   [Taxonomy](#)   [Books](#)

Search  for

☐ 1: [AAB29001](#). Reports beta-amyloid-(1-4...[gi:455742] BLink, Links

LOCUS      AAB29001                      19 aa                      linear      PRI 18-FEB-1994  
 DEFINITION   beta-amyloid-(1-42), A beta-(1-42)=amyloid major component  
                  {N-terminal} [human, leptomenigeal blood vessels, Peptide Partial,  
                  19 aa].

ACCESSION   AAB29001  
 VERSION      AAB29001.1    GI:455742  
 DBSOURCE     accession [AAB29001.1](#)  
 KEYWORDS      .

SOURCE       Homo sapiens (human)  
     ORGANISM   Homo sapiens  
                  Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;  
                  Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE    1 (residues 1 to 19)  
     AUTHORS    Roher,A.E., Lowenson,J.D., Clarke,S., Woods,A.S., Cotter,R.J.,  
                  Gowing,E. and Ball,M.J.  
     TITLE       beta-Amyloid-(1-42) is a major component of cerebrovascular amyloid  
                  deposits: implications for the pathology of Alzheimer disease  
     JOURNAL     Proc. Natl. Acad. Sci. U.S.A. 90 (22), 10836-10840 (1993)  
     MEDLINE    [94068497](#)  
     PUBMED     [8248178](#)

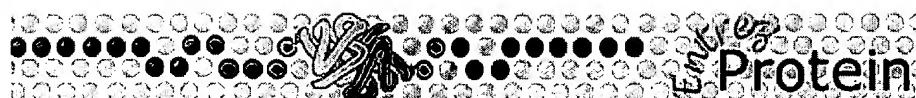
REMARK       GenBank staff at the National Library of Medicine created this  
                  entry [NCBI gibbsq 141019] from the original journal article.  
 COMMENT      Method: direct peptide sequencing.

FEATURES                      Location/Qualifiers  
     source                      1..19  
                                     /organism="Homo sapiens"  
                                     /db\_xref="taxon:9606"  
     Protein                      1..19  
                                     /product="beta-amyloid-(1-42)"  
                                     /name="amyloid major component"  
                                     /note="A beta-(1-42)"

ORIGIN  
     1 daefrhds gy evhhqklvf  
 //

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Boo

Search Protein

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

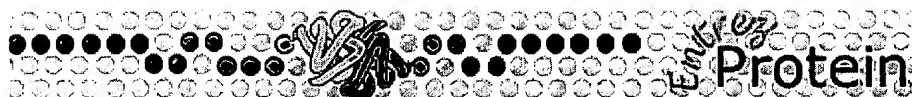
☐ 1: AAB56103. Reports Sequence 1 from p...[gi:2097271]

BLink, Links

LOCUS AAB56103 16 aa linear PAT 14-MAY-1997  
DEFINITION Sequence 1 from patent US 5605811.  
ACCESSION AAB56103  
VERSION AAB56103.1 GI:2097271  
DBSOURCE accession [AAB56103.1](#)  
KEYWORDS .  
SOURCE Unknown.  
ORGANISM Unknown.  
Unclassified.  
REFERENCE 1 (residues 1 to 16)  
AUTHORS Seubert,P.A., Schenk,D.B. and Fritz,L.C.  
TITLE Methods and compositions for monitoring cellular processing of  
beta-amyloid precursor protein  
JOURNAL Patent: US 5605811-A 1 25-FEB-1997;  
FEATURES Location/Qualifiers  
source 1..16  
/organism="unknown"  
ORIGIN  
1 gsgltnikte eisevk  
//

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10



Entrez

PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

Books

Search Protein  for 

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

default

Show:

20

Send to

File

Get Subsequence

Feat

☐ 1: AAB56104. Reports Sequence 2 from p...[gi:2097272]

BLink, Links

LOCUS AAB56104 17 aa linear PAT 14-MAY-1997  
 DEFINITION Sequence 2 from patent US 5605811.  
 ACCESSION AAB56104  
 VERSION AAB56104.1 GI:2097272  
 DBSOURCE accession AAB56104.1  
 KEYWORDS .  
 SOURCE Unknown.  
 ORGANISM Unknown.  
 Unclassified.  
 REFERENCE 1 (residues 1 to 17)  
 AUTHORS Seubert,P.A., Schenk,D.B. and Fritz,L.C.  
 TITLE Methods and compositions for monitoring cellular processing of  
 beta-amyloid precursor protein  
 JOURNAL Patent: US 5605811-A 2 25-FEB-1997;  
 FEATURES Location/Qualifiers  
 source 1..17  
 /organism="unknown"  
 ORIGIN  
 1 ysgltnikte eisevkm  
 //

[Disclaimer](#) | [Write to the Help Desk](#)  
[NCBI](#) | [NLM](#) | [NIH](#)

Nov 8 2004 13:44:10

## Analysis of the Transcriptional Unit Encoding the Genes for Rubredoxin (*rub*) and a Putative Rubredoxin Oxidoreductase (*rbo*) in *Desulfovibrio vulgaris* Hildenborough

MICHAEL J. BRUMLIK AND GERRIT VOORDOUW\*

Division of Biochemistry, Department of Biological Sciences, The University of Calgary, Calgary, Alberta T2N 1N4, Canada

Received 28 March 1989/Accepted 6 June 1989

The nucleotide sequence of a 2.0-kilobase-pair *Eco*RI restriction fragment upstream from the gene (*rub*, 162 base pairs) encoding rubredoxin from *Desulfovibrio vulgaris* Hildenborough indicates that it is part of a larger transcriptional unit, containing an additional 378-base-pair open reading frame which terminates 16 nucleotides from the translational start of the *rub* gene and could encode a polypeptide of 14 kilodaltons (kDa). Northern (RNA) blotting of RNA isolated from both *D. vulgaris* Hildenborough and *Escherichia coli* TG2 transformed with plasmid pJK29, which contains both genes on a 1.1-kilobase-pair *Sal*I insert, confirms that the genes for this 14-kDa polypeptide and rubredoxin are present on a single transcript of 680 nucleotides. Strong evidence that the 14-kDa polypeptide is also a redox protein is provided by the fact that its NH<sub>2</sub> terminus is homologous to desulfiredoxin, which has been isolated from *D. gigas* as a small dimeric redox protein (36 amino acids per monomer), coordinating two iron atoms. Since rubredoxin is a potential redox partner for the 14-kDa protein, it has been tentatively named rubredoxin oxidoreductase, produced by the *rbo* gene. Southern blotting indicates that the *rbo-rub* operon is present in several species and strains of sulfate-reducing bacteria.

Rubredoxins are small, electron-carrying proteins (molecular mass, 6 kilodaltons [kDa]), which are found in the cytoplasm of several anaerobic bacteria, e.g., *Desulfovibrio gigas* (8), *D. vulgaris* Hildenborough (7), *D. desulfuricans* (15), *Thermodesulfobacterium commune* (24), *Clostridium pasteurianum* (36), *Peptostreptococcus elsdenii* (4), and *Peptococcus aerogenes* (3). The amino acid sequences of these rubredoxins have been determined (3, 4, 7, 8, 15, 36), and the three-dimensional structures of rubredoxins from *C. pasteurianum*, *D. vulgaris*, *D. gigas*, and *D. desulfuricans*, which has a very low molecular mass (only 5.2 kDa) have been solved (2, 13, 14, 32, 41-43). The redox center of rubredoxin consists of a single iron atom (redox potential, -50 to 0 mV), coordinated to four cysteinyl sulfurs. This redox potential is relatively high, since dissimilatory sulfate reduction by *Desulfovibrio* species requires electrons at -400 to -200 mV (17, 18, 23, 27), and it is not clear, therefore, which electron transfer reaction is catalyzed by rubredoxin. Several attempts have been made to define the redox partners of rubredoxin by biochemical experiments. By using the rubredoxin-mediated reduction of eucaryotic cytochrome *c* by NADH as an assay, an NADH-rubredoxin oxidoreductase was (partially) purified from *D. gigas* (16) and *C. acetobutylicum* (25). The *Clostridium* enzyme was found to have flavin adenine dinucleotide as the prosthetic group and to consist of a single subunit with a molecular mass of 41 kDa (25). The *D. gigas* enzyme shows specificity for rubredoxin from *D. gigas* ( $K_m = 6.2 \times 10^{-6}$  M) relative to rubredoxins from *D. vulgaris* ( $K_m = 5.3 \times 10^{-5}$  M) and *C. pasteurianum* ( $K_m = 1.0 \times 10^{-4}$  M), which show 71 and 63% sequence identity, respectively, with *D. gigas* rubredoxin. The subunit molecular mass and nature of the cofactor have not been reported for the *D. gigas* enzyme, and it has not been isolated from *D. vulgaris*.

A different approach to the identification of possible redox

partners for rubredoxin, which makes use of the fact that the gene encoding rubredoxin (*rub*) from *D. vulgaris* Hildenborough was recently cloned from a  $\lambda$  library of the *D. vulgaris* chromosome and its nucleotide sequence was determined (37), is taken here. No typical promoter consensus sequence (29) was found in the region immediately upstream from the *rub* gene, which instead appears to contain the 3' end of another reading frame, indicating that the *rub* gene may be part of a larger transcriptional unit. Since at least one gene encoding a redox protein interacting with rubredoxin could be present on this larger transcript, the region upstream from the *rub* gene is characterized in detail in the present paper.

### MATERIALS AND METHODS

**Strains, vectors, and media.** The bacterial strains, plasmids, and cloning vectors used in this work are described in Table 1. For the isolation of RNA, *D. vulgaris* Hildenborough was grown anaerobically in a medium made up from the following solutions, described by Pfennig et al. (26): solution 1 (1 liter, kept under 90% N<sub>2</sub>-10% CO<sub>2</sub>), solution 2 (1 ml), solution 3 (1 ml), solution 4 (30 ml), solution 5 (3 ml), 15% (wt/vol) of sodium lactate (10 ml), solution 8 (1 ml), solution 9 (0.1 ml), and Wolin vitamins (10 ml) (44). This medium allows a high growth rate of *D. vulgaris*, and RNA can successfully be isolated from cells grown for 16 h at 30°C at a 5% (vol/vol) inoculum. Chromosomal DNA, isolated from 15 different species and strains of sulfate-reducing bacteria (see Table 2) after growth on Postgate medium C (27), was a gift from Helen Kent, AFRC Unit of Nitrogen Fixation, University of Sussex, Brighton, England. DNA from *D. vulgaris* Miyazaki was a gift from T. Yagi, Department of Chemistry, Shizuoka University, Shizuoka, Japan, and DNA from *D. salaxigens* NCIMB 8365 was donated by D. W. S. Westlake, Department of Microbiology, University of Alberta, Edmonton, Canada. *Escherichia coli* TG2 was grown in TY medium (22) containing 10 g of tryptone, 5

\* Corresponding author.



TABLE 1. Bacterial strains and DNA vectors used in this study

Strain or vector	Genotype, phenotype, comments, reference
<b>Strains</b>	
<i>D. vulgaris</i> subsp. <i>vulgaris</i> Hildenborough <sup>a</sup>	NCIMB 8303; isolated from clay soil near Hildenborough, U.K. (27)
<i>E. coli</i> TG2 <sup>b</sup>	$\Delta(lac-pro) supE thi hsdM hsdR recA F' (traD36 proAB^+ lacZ\Delta M15^F)$ ; from T. J. Gibson.
<b>Vectors</b>	
pUC8, pUC9	Amp <sup>r</sup> (35)
pJK15	This study (Fig. 1)
pJK29	This study (Fig. 1)
pRbo1	This study (Fig. 1)
pRub1	This study (Fig. 1)

<sup>a</sup> Abbreviated as *D. vulgaris* Hildenborough. A bacteriophage  $\lambda$  gene library has been constructed for this organism (37).

<sup>b</sup> Constructed from *E. coli* JM101 [ $\Delta(lac-pro) supE thi F' (traD36proAB^+ lacZ\Delta M15^F)$ ] by T. J. Gibson and M. D. Biggin, Laboratory of Molecular Biology, Medical Research Council Centre, Cambridge, U.K.

g of yeast extract, and 5 g of NaCl per liter of water at pH 7.4.

**Biochemical reagents.** All enzymes were obtained from Pharmacia, Inc., with the exception of calf alkaline phosphatase, which was from Boehringer Mannheim Biochemicals. The radioisotopes [ $\alpha$ -<sup>35</sup>S]dATP (400 Ci/mmol; 10 mCi/ml), [ $\alpha$ -<sup>32</sup>P]dATP (3,000 Ci/mmol; 10 mCi/ml), and [ $\gamma$ -<sup>32</sup>P]ATP (3,000 Ci/mmol; 10 mCi/ml) were purchased from Amersham Corp. and were used for dideoxynucleotide sequencing, nick translation, and 3' and 5' end labeling, respectively. Ficoll 400 was purchased from Pharmacia, Inc. Polyvinylpyrrolidone (molecular weight 40,000), bovine serum albumin (fraction V), molecular biology grade sodium

dodecyl sulfate (SDS), bakers' yeast tRNA, salmon sperm DNA (sodium salt), and dextran sulfate (molecular weight 500,000) were purchased from Sigma Chemical Co. Low- and high-gelling-temperature (LGT and HGT) agarose were obtained from Bethesda Research Laboratories, Inc. Nitrocellulose and Hybond-N hybridization transfer membranes were obtained from Schleicher & Schuell, Inc., and Amersham, respectively. All other reagent grade chemicals were purchased from either Sigma or Fisher Scientific Co.

**DNA cloning.** Several recombinant bacteriophages carrying the *rub* gene were isolated from a  $\lambda$  library, as described previously (37). These clones cover 35 kilobase pairs (kb) of the *D. vulgaris* Hildenborough chromosome, and an *Eco*RI restriction map of a part of this region is shown in Fig. 1. A 2.0-kb *Eco*RI fragment and a 1.1-kb *Sal*I fragment (Fig. 1) were cloned into the *Eco*RI and *Sal*I sites of pUC8 (35), generating plasmids pJK15 and pJK29, respectively, which were purified as described elsewhere (40).

**Shotgun nucleotide sequencing.** The procedure followed for shotgun nucleotide sequencing was essentially that outlined by Bankier and Barrell (5). Plasmid pJK15 was sonicated, and the resulting fragments were end repaired and size fractionated by gel electrophoresis on 1% (wt/vol) LGT agarose. The 400- to 1,000-base-pair (bp) fraction was excised and isolated from the gel and ligated to the replicative form of M13mp8 (21), digested with *Sma*I and calf alkaline phosphatase (5). After being transfected into competent,  $CaCl_2$ -treated *E. coli* TG2 cells, the ligation mixtures were spread onto TY plates with top agar, containing isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal) (5). Single-stranded DNA was isolated from white recombinant phage plaques by using a 1.5-ml miniprep procedure (5). The purified DNAs (2  $\mu$ l) were spotted on a nitrocellulose filter, which was subsequently baked at 80°C for 1 h under vacuum. The 2.0-kb *Eco*RI insert from pJK15 was isolated by LGT agarose gel electrophoresis, radiolabeled by nick translation

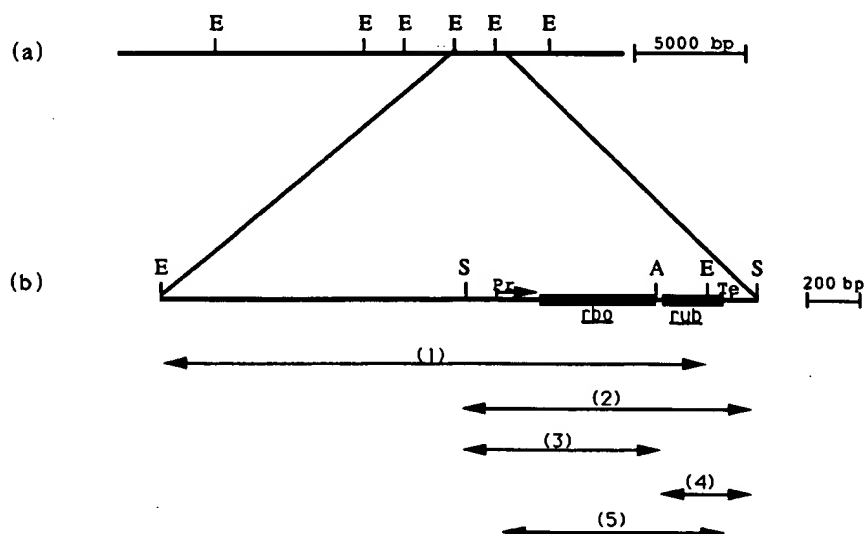


FIG. 1. Survey of DNA containing the *rub* gene of *D. vulgaris* Hildenborough. (a) Location of *Eco*RI (E) restriction sites as determined earlier (37). (b) Map of a 2,234-bp *Eco*RI-*Sal*I region of DNA for which the nucleic acid sequence was determined. The location of restriction sites for *Eco*RI, *Sal*I (S), and *Ava*I (A), the coding regions for the genes encoding rubredoxin (*rub*) and the putative rubredoxin oxidoreductase (*rbo*), and the location of promoter (Pr) and transcription terminator (Te) sequences, are indicated. The following plasmids (inserts) were constructed: 1, pJK15 (2.0 kb EE); 2, pJK29 (1.1 kb SS); 3, pRbo1 (746 bp AS) and 4, pRub1 (347 bp AS). The position of the nucleic acid sequence shown in Fig. 3 is indicated in region 5.

(20), and denatured by boiling. The filter was prehybridized in  $6\times$  SSC ( $1\times$  SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.2])– $10\times$  Denhardt solution (20)–0.5% (wt/vol) SDS for 15 min at 68°C. The 2.0-kb DNA probe was then added to the prehybridization solution, and hybridization was continued for 16 h at 68°C. After hybridization, the filter was washed in  $6\times$  SSC–0.5% (wt/vol) SDS at 68°C for 1 h. The filter was then dried and wrapped in Saran wrap, and positive clones were identified by autoradiography. These were then sequenced by the dideoxy-chain termination procedure developed by Sanger et al. (31), as detailed by Bankier and Barrell (5). The sequencing data were processed and analyzed by using the programs of Staden and McLachlan (33, 34).

**Construction of gene-specific probes.** The completed nucleotide sequence indicated the presence of a unique *Ava*I site in the 19-bp region separating the *rho* and *rub* genes (Fig. 1; see also Fig. 3). Plasmid pJK29 was digested with *Ava*I, and the digested DNA was end repaired with the Klenow fragment of DNA polymerase I to generate blunt ends (20). Following inactivation of the polymerase at 68°C, pJK29 was further digested with *Sal*I, resulting in the excision of both the *rho* and *rub* genes on 746- and 347-bp fragments of DNA, respectively; these were isolated by electrophoresis on 1.5% (wt/vol) LGT agarose. The purified fragments were ligated into pUC8, previously digested with *Sal*I and *Sma*I, to give plasmids pRbo1 and pRub1, respectively. The insert of pRbo1 was gel isolated following digestion with *Hind*III and *Eco*RI as a 767-bp *rho* gene-specific probe, whereas the presence of an *Eco*RI site near the 3' end of the *rub* gene (Fig. 1; see also Fig. 3) allowed the isolation of a 162-bp *rub* gene-specific probe by digestion of pRub1 with *Eco*RI and gel electrophoresis.

**RNA isolation and Northern blotting.** RNA was isolated from cultures of *D. vulgaris* Hildenborough and *E. coli* TG2 by the hot-phenol extraction method (11) and stored as the ethanol precipitate at –20°C. Samples of RNA were collected by centrifugation, washed with 70% (vol/vol) ethanol, dried under vacuum, and redissolved in 20  $\mu$ l of loading buffer, prepared as described elsewhere (10). For Northern (RNA) blotting, RNA samples were electrophoresed on gels containing 1.0% (wt/vol) HGT agarose in  $1\times$  MOPS buffer ( $10\times$  MOPS buffer is 0.2 M 3-*N*-morpholinopropanesulfonic acid, 10 mM EDTA, and 50 mM sodium acetate [pH 7.0]) containing 2% (vol/vol) formaldehyde and then blotted onto a Hybond-N hybridization transfer membrane. Following transfer, the membrane was exposed to shortwave UV light to cross-link the RNA to the filter. Blots were prehybridized for 4 h at 42°C in a solution containing 0.75 M NaCl, 0.075 M sodium citrate (pH 7.0), 50% (vol/vol) formamide, 1% (wt/vol) SDS, 0.1% (wt/vol) Ficoll 400, 0.1% (wt/vol) polyvinylpyrrolidone, 0.1% (wt/vol) bovine serum albumin, and 0.16 mg of boiled bakers' yeast tRNA per ml. Following prehybridization, the blots were hybridized at 42°C for 16 h in the solution described above, also containing 10% (wt/vol) dextran sulfate as a hybridization enhancer, with a nick-translated probe derived from either plasmid pRbo1 or pRub1. Following hybridization, the blots were washed twice for 5 min at room temperature with 100 ml of 0.3 M NaCl–0.06 M Tris hydrochloride (pH 8.0)–0.002 M EDTA, then twice for 15 min at 60°C with 100 ml of the same solution with 0.5% (wt/vol) of SDS added, and, finally, twice for 15 min at room temperature with 0.003 M Tris base, after which the filters were dried and autoradiographed.

**S1 nuclease mapping.** A probe covering the region upstream from the *rbo* gene was prepared by digestion of

pJK29 with *Eco*RI and calf alkaline phosphatase and gel isolation of the 913-bp *Eco*RI fragment, containing the relevant *Sal*I–*Eco*RI fragment. The probe was 5' end labeled by adding T4 polynucleotide kinase (1  $\mu$ l; 10 U/ $\mu$ l) and [ $\gamma$ -<sup>32</sup>P]ATP (2.5  $\mu$ l; 10  $\mu$ Ci/ $\mu$ l) in the presence of 50 mM Tris hydrochloride (pH 7.6)–10 mM MgCl<sub>2</sub>–3 mM dithiothreitol–0.1 mM EDTA in a total volume of 10  $\mu$ l at room temperature for 1 h. The labeled probe was phenol extracted, ethanol precipitated, and dissolved in 20  $\mu$ l of 80% (vol/vol) formamide–20 mM PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid)] (pH 6.5)–400 mM NaCl (hybridization buffer). RNA (100  $\mu$ g) was dissolved in 40  $\mu$ l of hybridization buffer and incubated with the probe at 44°C for 12 h. Following the addition of 240  $\mu$ l of S1 nuclease buffer (300 mM NaCl, 18 mM sodium acetate [pH 4.6], 10 mM ZnSO<sub>4</sub>) and 2  $\mu$ l of S1 nuclease (384,000 U/ $\mu$ l), the hybridization solution was incubated at room temperature for 30 min. The reaction was terminated by the addition of 10  $\mu$ l of denatured bakers' yeast tRNA (1  $\mu$ g/ $\mu$ l), and then phenol extraction and ethanol precipitation of the DNA-RNA hybrids for 1 h at –70°C were carried out. The vacuum-dried samples were dissolved in 10  $\mu$ l of formamide dye mixture, prepared as described by Bankier and Barrell (5), and heated at 80°C for 5 min before being loaded on either a 4 or 6% (wt/vol) denaturing acrylamide gel (5), which was run at 39 W for 2 to 3 h. A partial *Hin*II digest of plasmid pUC8, labeled by filling the ends with Klenow polymerase in the presence of dGTP, dCTP, dTTP, and [ $\alpha$ -<sup>32</sup>P]dATP, was used as a molecular size marker (1,613, 913, 517, 396, 140, 75, and 65 nucleotides).

**Southern blotting.** Samples of genomic DNA (3 to 10  $\mu$ g) from 17 different species and strains of sulfate-reducing bacteria were digested with *Eco*RI, electrophoresed on a 0.7% (wt/vol) HGT agarose gel as described elsewhere (40), and blotted on Hybond-N hybridization transfer membrane. The DNA was bound to the membrane by UV cross-linking as described above, and the blots were prehybridized for 1 h at 42°C with a solution (pH 7.4) containing 50% (vol/vol) formamide, 1 M NaCl, 0.1% (wt/vol) sodium PP<sub>i</sub>, 0.2% (wt/vol) polyvinylpyrrolidone, 0.2% (wt/vol) bovine serum albumin, 0.2% (wt/vol) Ficoll 400, 10% (wt/vol) dextran sulfate, and 0.175 mg of denatured salmon sperm DNA per ml. A nick-translated pRub1 probe (20) was then added, and hybridization was continued for 16 h. The Southern blots were washed similarly to the Northern blots, dried, and autoradiographed. The pRub1 probe was removed by washing with 0.5 M NaOH–1.5 M NaCl for 30 min and neutralizing for 30 min with 1 M Tris hydrochloride (pH 8.0)–1.5 M NaCl. Removal of the probe was confirmed by autoradiography, after which the blots were rehybridized with the nick-translated pRbo1 probe.

## RESULTS

**Nucleotide sequence upstream from *rub*.** The nucleotide sequence of the 2.0-kb *Eco*RI fragment, containing the region upstream from the *rub* gene, was determined by the random cloning and dideoxy sequencing procedure of Bankier and Barrell (5), and these data were combined with the sequence of the *rub* gene (37) to obtain a sequence of 2,234 nucleotides (nt) extending from an *Eco*RI to a *Sal*I site (Fig. 1b). An identification of possible coding regions with the aid of the codon probability method of Staden and McLachlan (34) in this 2.2-kb sequence is shown in Fig. 2. Apart from the *rub* gene, this analysis indicates the presence of two other plausible genes in frames a and b. The amino acid sequences derived from these possible genes were compared

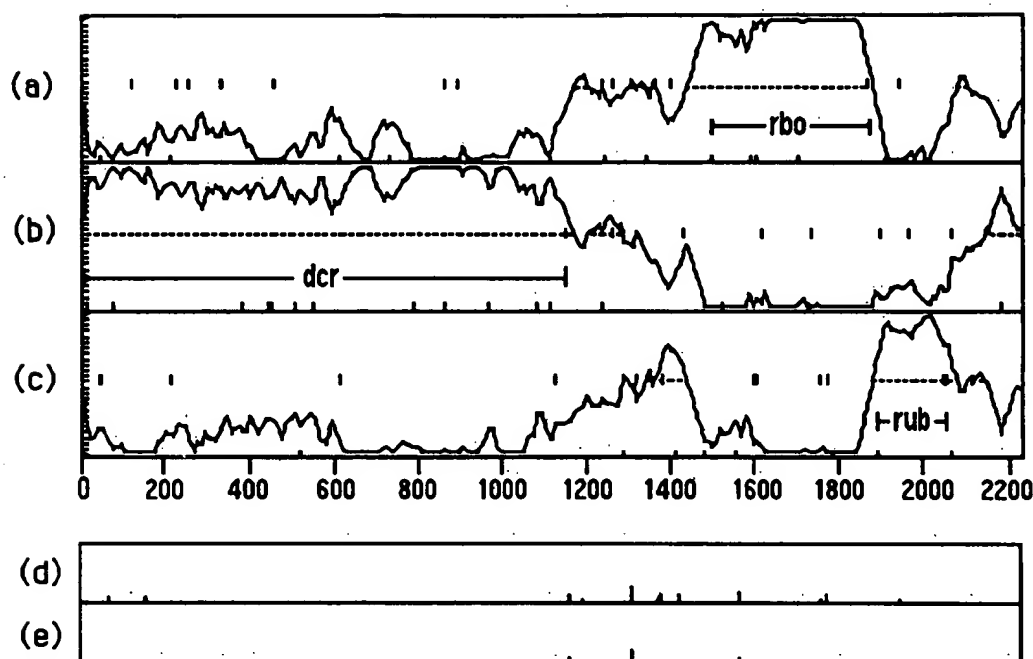


FIG. 2. Statistical analysis of the 2,234-bp region outlined in Fig. 1b. (a to c) The probability that a stretch of sequence (25 bases) is coding is calculated and plotted for each of the three reading frames a, b, and c (36). A codon usage table, which includes all codons of structural genes from *D. vulgaris* Hildenborough, was used as the standard in the calculation. Methionine (ATG) start codons are indicated on the base line, and stop codons are indicated at the half level of each frame. The coding regions for a putative rubredoxin oxidoreductase (*rbo*), a putative *D. vulgaris* chemoreceptor (*dcr*), and rubredoxin (*rub*) are indicated by the calculation as shown in frames a to c, respectively. (d and e) Search for *E. coli* promoters. Sequences with homology to the consensus  $-35$  (TTGACA) and  $-10$  (TATAAT) *E. coli* promoter sequence are indicated in frames d and e, respectively. The scale is in base pairs.

with sequences in the Bionet data base, which indicated unique and extensive homologies with, respectively, desulfiredoxin of *D. gigas* (9) and the aspartate and serine chemoreceptors of *Salmonella typhimurium* (30) and *E. coli* (6), respectively. The amino acid sequence that can be derived from the sequenced portion of the *dcr* gene (nt 1 to 1158) lacks approximately 140  $\text{NH}_2$ -terminal amino acids, and further nucleic acid sequencing is required for the completion of the sequence of the putative *Desulfovibrio* chemoreceptor protein. The homology of the *dcr* gene product with these two known chemotactically active proteins will therefore not be further discussed here, allowing a focus on the gene immediately preceding the *rub* gene. In view of the homology with desulfiredoxin, as discussed below, the upstream gene appears to also encode a redox protein and is tentatively referred to as rubredoxin oxidoreductase, the product of the *rbo* gene.

The nucleotide sequence of the *rub* gene and its upstream region (nt 1301 to 2140) is shown in Fig. 3. Each nucleotide was determined four times on average, at least once on each strand. The gene encoding rubredoxin (nt 1896 to 2057) and the putative *rbo* gene predicted by the codon probability calculations in Fig. 2a (nt 1499 to 1879) have been translated into amino acid sequences. Each gene is preceded by a plausible ribosome-binding site. A search for *E. coli* promoters (Fig. 2d and e) indicates the presence of a promoter consensus sequence ( $-35$ ,  $-10$ ) upstream from these two genes, and a potential promoter has been indicated in Fig. 3. A hairpin-loop-forming structure of nine G-C base pairs, which may serve as a transcription terminator, is present immediately downstream from the *rub* gene. Thus, an analysis of the nucleic acid sequence indicates that the two genes

may form an operon. Direct experimental evidence for the presence of both genes on a single transcript is presented below.

**Northern blotting and S1 nuclease mapping.** Northern blots of RNA isolated from both *D. vulgaris* and *E. coli* TG2(pJK29) indicate that a single transcript of approximately 680 nucleotides hybridizes with both the pRb01 and the pRub1 probes (Fig. 4). The 1.1-kb insert of pJK29 is in the correct orientation for transcription of the *rbo* and *rub* genes from the *E. coli* *lac* promoter present on the pUC vector. However, a transcript originating from the *lac* promoter and containing both the *rbo* and *rub* genes would be approximately 1,100 nt long, and this size is not consistent with the data in Fig. 4C. The observation of similarly sized transcripts in *D. vulgaris* and *E. coli* indicates that the *D. vulgaris* promoter controlling the transcription of these two genes functions in *E. coli* and may thus resemble the *E. coli* consensus. The observed size of the transcript is sufficiently large to accommodate both the *rbo* and the *rub* genes. Assuming that the transcript terminates at position 2110, on the 3' side of the hairpin indicated in Fig. 3, the transcriptional start site is placed in the vicinity of position 1430, which is 70 nt upstream from the translational start of the *rbo* gene. The transcriptional start site was next defined more precisely by S1 nuclease mapping. Use of the 913-bp probe gives rise to a major protected fragment of  $600 \pm 50$  nt (average of three experiments), while minor protected fragments of variable, smaller sizes are also observed (Fig. 5). The 600-nt fragment ends at nt 2041 (Fig. 3), indicating a transcriptional start site at  $nt\ 1441 \pm 50$ . The presence of a promoter sequence with reasonable homology to the *E. coli* consensus at nt 1355 to 1380 (Fig. 3) suggests a potential start

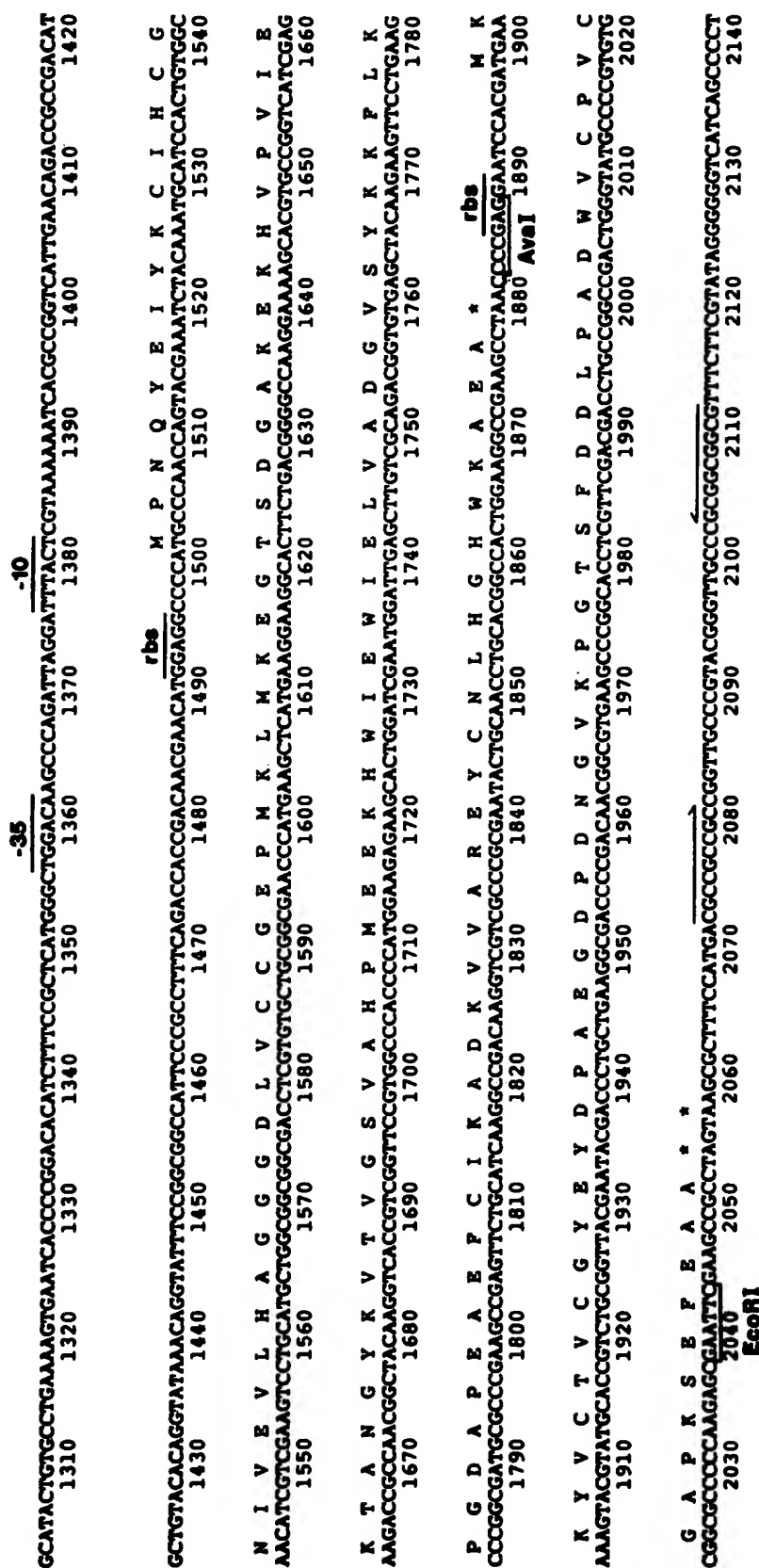


FIG. 3. Nucleotide sequence of the *rbo-rub* operon. The numbering of the sequence is the same as in Fig. 1 and 2. The coding regions of the *rbo* (nt 1499 to 1879) and *rub* (nt 1896 to 2057) genes have been translated with the single-letter amino acid code. Translation stop codons are designated by asterisks. The ribosome-binding sites (rbs) and a plausible promoter (—35, —10) are indicated. A G+C-rich stem-and-loop structure (—) which could serve as a transcription termination signal is shown.

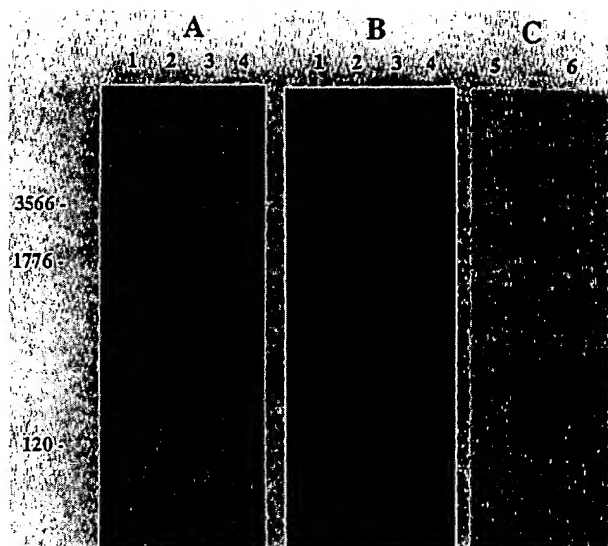


FIG. 4. Northern blotting of total RNA isolated from *D. vulgaris* Hildenborough and *E. coli* TG2(pJK29). (A) Increasing amounts of *D. vulgaris* RNA (25, 50, 75, and 100  $\mu$ g) were loaded in lanes 1 to 4, respectively. The blot was hybridized to the pRbo1 probe and autoradiographed for 5 days at  $-70^{\circ}\text{C}$ . The size markers, 25S rRNA (3,566 bases), 16S rRNA (1,776 bases), and 5S rRNA (120 bases), were visualized by fluorography of the blot following transfer of the ethidium bromide-stained gel. (B) The same blot as in panel A was incubated for 2 min in boiling distilled water to dissociate the pRbo1 probe. Following autoradiography to confirm the dissociation of this probe, the blot was rehybridized to the pRub1 probe. Autoradiography was carried out for 7 days at  $-70^{\circ}\text{C}$ . (C) *D. vulgaris* RNA (25  $\mu$ g) and RNA isolated from *E. coli* TG2 transformed with plasmid pJK29 (100  $\mu$ g) were loaded in lanes 5 and 6, respectively. Following electrophoresis, the blot was incubated with the pRbo1 probe. Autoradiography was performed for 14 days at  $-70^{\circ}\text{C}$ . Use of the pRub1 probe gave identical results (not shown).

site in the vicinity of nt 1390, which is within the range predicted by the S1 nuclease mapping results. The smaller (<600-nt) fragments do not map to the same position and are presumed to be artifacts arising from preferential S1 nuclease cleavage (12).

**Southern blotting.** Southern blots of chromosomal DNA digested with *Eco*RI from 17 different species and strains of sulfate-reducing bacteria, including *D. vulgaris* Hildenborough, were hybridized with the inserts from plasmids pRub1 and pRbo1 as probes. The results obtained are shown in Fig. 6A and B, respectively, as well as in Table 2. These results are discussed in more detail below.

## DISCUSSION

**Homology of the *rbo* gene product with desulforedoxin.** Desulforedoxin is a small redox protein, which has been isolated from *D. gigas* but has so far not been found in other sulfate-reducing bacteria. Its polypeptide chain is only 36 amino acids long and has been determined by protein sequencing (9). As discussed by Le Gall et al. (18), the protein is isolated as a dimer (molecular mass, 7.9 kDa; 3.9 kDa per subunit) with two bound Fe atoms as redox centres and has rubredoxinlike spectroscopic properties. The product of the *rbo* gene (378 nt) is much larger than desulforedoxin (126 amino acids; molecular mass, 14 kDa). A comparison of the two amino acid sequences shows that the homology resides

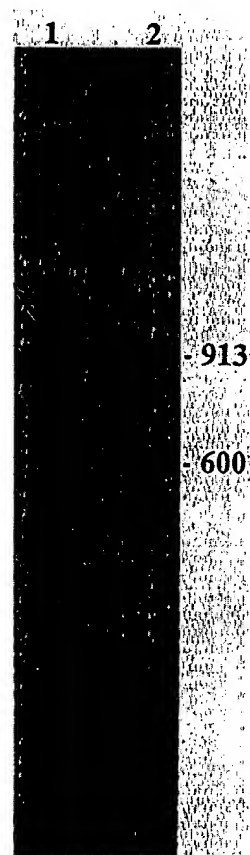


FIG. 5. S1 nuclease mapping of the transcriptional start site of the *rbo-rub* operon. Lanes: 1, 913-nt probe control; 2, *D. vulgaris* RNA (100  $\mu$ g) hybridized with the 913-nt probe and treated with S1 nuclease; a major protected fragment of 600 nt is indicated. The size of this fragment was derived from the positions of pUC8  $\times$  *Hinf*I markers (not shown). Autoradiography was performed for 4 weeks at  $-70^{\circ}\text{C}$ .

entirely at the  $\text{NH}_2$  terminus (Fig. 7). Thus, of the first 36 amino acids of the *rbo* gene product, 19 are identical to desulforedoxin, whereas conservative amino acid changes are frequently found in the nonidentical positions. The four cysteine residues (C-9, C-12, C-28, and C-29), which have been proposed to coordinate to the iron (18), are among the conserved amino acid residues. Another interesting region of homology comprises the three glycine residues (G-22, G-23, G-24), which must represent an important flexible (e.g., loop) region of the molecule. The high degree of homology indicates that at its  $\text{NH}_2$  terminus, the *rbo* gene product must have a 4-kDa desulforedoxin domain, which has been fused to a larger polypeptide of 10 kDa. This situation is very reminiscent to that in [Fe] hydrogenase of *D. vulgaris* Hildenborough, which is known to have three iron-sulfur clusters as prosthetic groups coordinated by cysteine residues of the large (46-kDa) subunit. The  $\text{NH}_2$  terminus of this large subunit is homologous to bacterial 8Fe-8S ferredoxin (38), indicating that two electron-transferring 4Fe-4S clusters are likely to be present in this part of the molecule in a structure resembling that of ferredoxin (1), while a third unique hydrogen-binding cluster could be coordinated by some of the 10 cysteine residues found in the 40-kDa COOH-terminal part of the molecule. It was proposed, therefore, that the hydrogenase gene originated from a

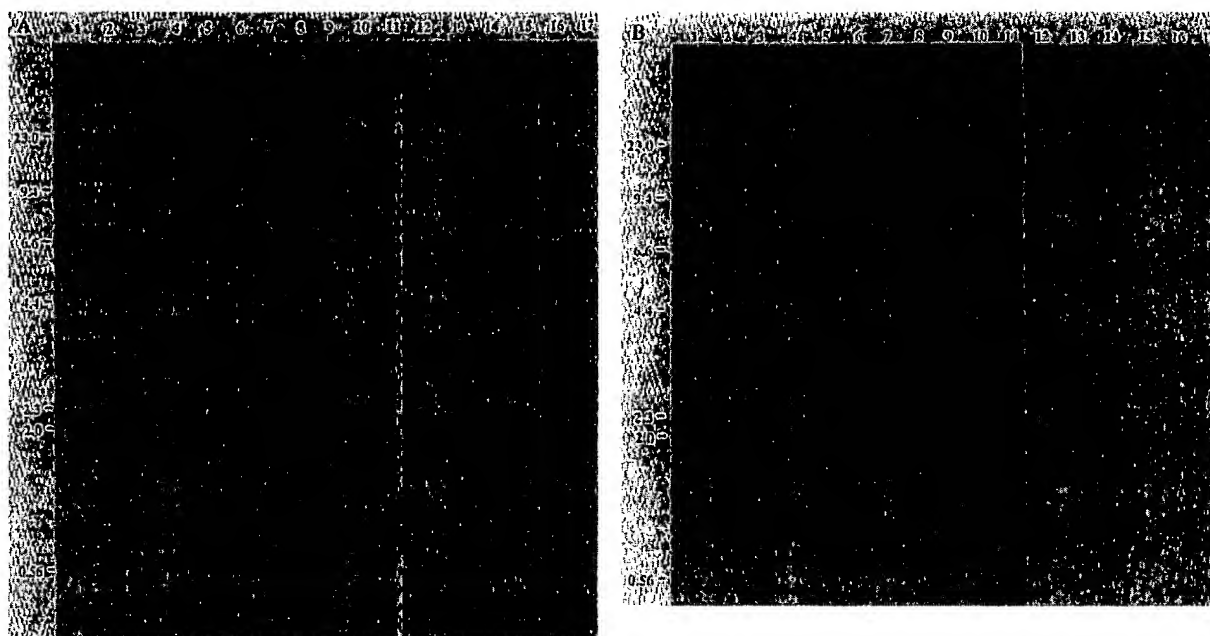


FIG. 6. Southern blotting of *Eco*RI-digested chromosomal DNA (5 to 10  $\mu$ g) from 17 species and strains of sulfate-reducing bacteria. Lanes: 1, *D. vulgaris* Hildenborough; 2, *D. vulgaris* Wandle; 3, *D. vulgaris* Brockhurst Hill; 4, *D. vulgaris* Groningen; 5, *D. vulgaris* Miyazaki; 6, *D. vulgaris* subsp. *oxamicus* Monticello; 7, *D. desulfuricans* Norway; 8, *D. desulfuricans* Teddington R; 9, *D. desulfuricans* El Agheila Z; 10, *D. desulfuricans* Berre Sol; 11, *D. desulfuricans* Canet 41; 12, *D. gigas*; 13, *D. salexigens* British Guiana; 14, *D. salexigens* California; 15, *D. salexigens*; 16, *D. africanus* Walvis Bay; 17, *D. africanus* Bhengazi. (A) The blots were hybridized with the pRub1 probe (containing part of the rubredoxin gene from *D. vulgaris* Hildenborough) and subjected to autoradiography for 8 days at  $-70^{\circ}\text{C}$ . The probe was then dissociated from the blots by washing in 0.5 M NaOH–1.5 M NaCl and neutralized by washing with 0.5 M Tris hydrochloride (pH 7.4)–1.5 M NaCl. Complete dissociation of the pRub1 probe was confirmed by autoradiography. (B) The blots were then hybridized with the pRbo1 probe (containing the gene of a putative rubredoxin oxidoreductase from *D. vulgaris* Hildenborough) and subjected to autoradiography for 2.5 days at  $-70^{\circ}\text{C}$ . The positions of molecular size markers (bacteriophage  $\lambda$  DNA digested with *Hind*III) are indicated in kilobases.

TABLE 2. Hybridization of *Eco*RI-digested chromosomal DNA from 17 different sulfate-reducing bacteria with an *rub*-specific and an *rbo*-specific probe from *D. vulgaris* Hildenborough

Species	Strain	NCIMB no.	Size of hybridizing fragment (kb) for:	
			<i>rub</i>	<i>rbo</i>
<i>D. vulgaris</i> subsp. <i>vulgaris</i>	Hildenborough	8303	2.0	2.0
	Wandle	8306	2.0	2.0
	Brockhurst Hill	8306	2.0	2.0
	Groningen	11779	15.4	15.4
	Miyazaki		0.5	3.9
<i>D. vulgaris</i> subsp. <i>oxamicus</i>	Monticello 2	9442	0.5	1.8
<i>D. desulfuricans</i> subsp. <i>desulfuricans</i>	Norway 4	8310		
	Teddington R <sup>a</sup>	8312		7.8
	El Agheila Z	8318		
	Berre Sol	8388	0.5	2.8
	Canet 41	8393	0.5	
<i>D. gigas</i>		9332	1.2	
<i>D. salexigens</i>	British Guiana	8403		
	California 43:63	8364		
		8365		
<i>D. africanus</i>	Walvis Bay	8397	7.1	
	Bhengazi	8401	0.4	3.0

<sup>a</sup> Reclassified as *D. vulgaris* (28).

fusion of genes encoding an electron-transferring ferredoxin and a larger hydrogen-binding polypeptide (38). In the present case, the desulforedoxin domain coincides precisely with the 36 NH<sub>2</sub>-terminal amino acids.

Another example of a redox protein that could have arisen by gene fusion is rubrerythrin, which was recently isolated (19) and shown to contain both rubredoxinlike and hemerythrinlike redox centres. The putative *rbo* gene product is distinct from rubrerythrin in its molecular mass (14 versus 22 kDa) and amino acid composition. Also, preliminary protein sequence data on rubrerythrin (D. M. Kurtz, Jr., and J. Le Gall, personal communication) indicate that this protein does not resemble the *rbo* gene product.

Two possible modes of coordination of the two iron atoms by the eight cysteines of a desulforedoxin dimer have been considered by Le Gall et al. (18): the four cysteines of a single subunit could coordinate to the same iron, in which case dimer formation is achieved by noncovalent interactions of the two subunits, or cysteines from both subunits (e.g., C-9, C-12, and C-28 from one and C-29 from the other) could contribute to the coordination of the two irons, causing a partially covalent connection of the two subunits via the two Fe redox centres. In view of its homology with desulforedoxin, the functional form of the *rbo* gene product is also proposed to be a dimer. Only two additional cysteine residues (C-103 and C-115) are present in the remainder of the sequence of the *rbo* gene product (Fig. 7), which is insufficient for the coordination of a third Fe-containing redox centre (e.g., the coordination of a single Fe, a single

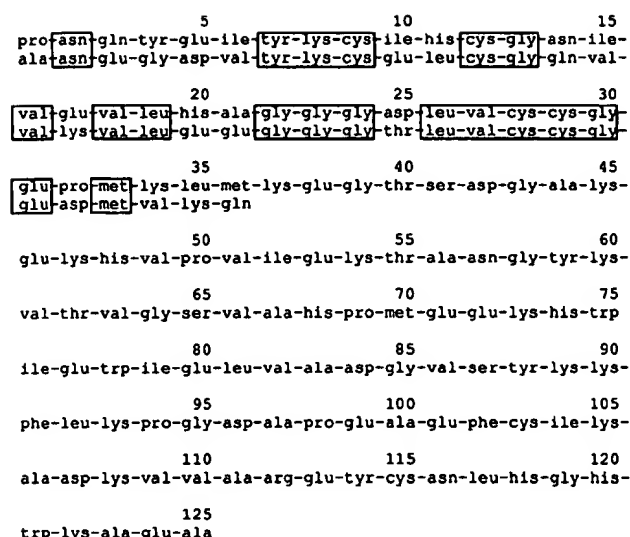


FIG. 7. Comparison of the amino acid sequence of the *rbo* gene product (Fig. 3) with that of desulfiredoxin (9). Regions of sequence identity are highlighted by boxes.

2Fe-2S cluster, or a single 4Fe-4S cluster requires four cysteines). However, a third redox centre could be present in the 14-kDa *rbo* gene product, if one assumes that the two subunits can donate both cysteine ligands to the same coordination site. The *rbo* gene product appears quite different from the NADH-rubredoxin oxidoreductase purified from *C. acetobutylicum* which was discussed in the Introduction. The subunit molecular mass is different (41 kDa for the *Clostridium* enzyme), and the search of the Bionet data base did not reveal homologies of the *rbo* gene product with flavin-binding proteins.

In analogy with the [Fe] hydrogenase gene, one could suggest that the *rbo* gene has arisen by fusion of the gene for desulfiredoxin with that encoding a 10-kDa redox protein containing a single redox centre. The observation that this gene forms a single operon with the gene encoding rubredoxin could mean that the two proteins are redox partners, but definitive proof for this proposal must await the purification and characterization of this novel redox protein.

**Distribution of the *rbo* gene in sulfate-reducing bacteria.** The *rub* and *rbo* genes are present on a similarly sized *EcoRI* fragment (Fig. 6) and may thus form an operon as in Fig. 3 in *D. vulgaris* Wandle, Brockhurst Hill, and Groningen. This is no surprise for the first two strains, which are practically identical to the Hildenborough strain (39). However, the Groningen strain is quite distinct from Hildenborough and was shown to lack a gene for [Fe] hydrogenase and to have only a low degree of homology in its gene for cytochrome *c*<sub>3</sub> (39). Four species of sulfate-reducing bacteria have homologous *rub* and *rbo* genes on different restriction fragments (Table 2; Fig. 6). This could indicate a separation of the two genes in these species or the presence of an additional *EcoRI* site in their *rbo-rub* operon. Finally, a homologous *rub* gene in the absence of a detectable, homologous *rbo* gene is found in three, whereas the converse is found in a single one of the species of sulfate-reducing bacteria examined. *D. gigas*, which has a rubredoxin with 71% sequence identity (37 identical residues in a total of 52) with the protein from *D. vulgaris* Hildenborough (36), the pRub1 probe appears sufficiently homologous for the detection of the *rub* gene on a 1.2-kb *EcoRI* fragment (Fig. 6A, lane 12; Table 2). However,

the 53% identity of *D. gigas* desulfiredoxin with the N terminus of the *D. vulgaris* *rbo* gene product (Fig. 7; 19 identical residues in a total of 36) appears insufficient for the detection of the desulfiredoxin gene in *D. gigas* (Fig. 6B, lane 12). The data in Fig. 6 should therefore not be interpreted in terms of the presence or absence of a gene encoding a desulfiredoxinlike polypeptide in species that fail to hybridize with the pRub1 probe. They indicate that the *rbo* gene is present in nine other species of sulfate-reducing bacteria and is spatially linked to the *rub* gene in at least four species. The *rbo* gene product could thus function as a redox protein in some but not all species of sulfate-reducing bacteria.

#### ACKNOWLEDGMENTS

M.J.B. acknowledges the support of Graduate Teaching and Graduate Research Assistantships from the University of Calgary. This work was supported by an establishment grant from the Alberta Heritage Foundation for Medical Research and an operating grant from the National Science and Engineering Research Council of Canada to G.V.

#### LITERATURE CITED

- Adman, E. T., L. C. Sieker, and L. H. Jensen. 1973. The structure of a bacterial ferredoxin. *J. Biol. Chem.* **248**:3987-3996.
- Adman, E. T., L. C. Sieker, L. H. Jensen, M. Bruschi, and J. Le Gall. 1977. A structural model of rubredoxin from *Desulfovibrio vulgaris* at 2 Å resolution. *J. Mol. Biol.* **112**:113-120.
- Bachmayer, H., A. M. Benson, K. T. Yasunobu, W. T. Garrard, and H. R. Whiteley. 1968. Non-heme iron proteins. IV. Structural studies of *Micrococcus aerogenes* (*Peptococcus aerogenes*) rubredoxin. *Biochemistry* **7**:968-996.
- Bachmayer, H., J. L. Peel, K. T. Yasunobu, and S. G. Mayhew. 1968. Non-heme iron proteins. V. The amino acid sequence of rubredoxin from *Peptostreptococcus elsdenii*. *J. Biol. Chem.* **243**:1024-1032.
- Bankier, A. T., and B. G. Barrell. 1983. Shotgun DNA sequencing, p. 1-34. In R. A. Flavell (ed.), *Techniques in the life sciences*, B5. Nucleic acid biochemistry. Elsevier Scientific Publishers Ireland Ltd., Shannon, Ireland.
- Boyd, A., K. Kendall, and M. Simon. 1983. Structure of the serine chemoreceptor in *Escherichia coli*. *Nature (London)* **301**:623-626.
- Bruschi, M. 1976. Non-heme iron proteins: the amino acid sequence of rubredoxin from *Desulfovibrio vulgaris*. *Biochim. Biophys. Acta* **434**:4-17.
- Bruschi, M. 1976. The amino acid sequence of rubredoxin from the sulfate-reducing bacterium *Desulfovibrio gigas*. *Biochem. Biophys. Res. Commun.* **70**:615-621.
- Bruschi, M., I. Moura, J. Le Gall, A. V. Xavier, and L. C. Sieker. 1979. The amino acid sequence of desulfiredoxin, a new type of non heme iron protein from *Desulfovibrio gigas*. *Biochem. Biophys. Res. Commun.* **90**:596-605.
- Davis, L. G., J. F. Battey, and M. P. Dibner. 1986. Basic methods in molecular biology, p. 143-146. Elsevier Science Publishing Co. Ltd., Amsterdam.
- Ebina, Y., and A. Nakazawa. 1983. Cyclic AMP-dependent initiation and p-dependent termination of colicin E1 gene transcription. *J. Biol. Chem.* **258**:7072-7078.
- Favaloro, J., R. Treisman, and R. Kamen. 1980. Transcription maps of polyoma virus-specific RNA: analysis by 2-dimensional nuclease S<sub>1</sub> gel mapping. *Methods Enzymol.* **65**:718-749.
- Frey, M., L. Sieker, F. Payan, R. Haser, M. Bruschi, G. Pepe, and J. Le Gall. 1987. Rubredoxin from *Desulfovibrio gigas*. A molecular model of the oxidized form of 1.4 Å resolution. *J. Mol. Biol.* **197**:525-541.
- Herriott, J. R., L. C. Sieker, L. H. Jensen, and W. Lovenberg. 1970. Structure of rubredoxin: an X-ray study to 2.5 Å resolution. *J. Mol. Biol.* **50**:391-406.



15. Hormel, S., K. A. Walsh, B. C. Prickril, K. Titani, J. Le Gall, and L. C. Sieker. 1986. Amino acid sequence of rubredoxin from *Desulfovibrio desulfuricans* strain 27774. *FEBS Lett.* 201:147-150.
16. Le Gall, J. 1968. Purification partielle et étude de la NAD: rubrédoxine oxydo-réductase de *D. gigas*. *Ann. Inst. Pasteur (Paris)* 114:109-115.
17. Le Gall, J., D. V. DerVartanian, and Harry D. Peck, Jr. 1979. Flavoproteins, iron proteins, and hemoproteins as electron-transfer components of the sulfate-reducing bacteria. *Curr. Top. Bioenerg.* 9:237-265.
18. Le Gall, J., J. J. G. Moura, H. D. Peck, Jr., and A. V. Xavier. 1982. Hydrogenase and other iron-sulfur proteins from sulfate-reducing and methane forming bacteria, p. 177-248. *In* T. G. Spiro (ed.), *Iron-sulfur proteins*. John Wiley & Sons, Inc., New York.
19. Le Gall, J., B. C. Prickril, I. Moura, A. V. Xavier, J. J. G. Moura, and B.-H. Huynh. 1988. Isolation and characterization of rubrerythrin, a non-heme iron protein from *Desulfovibrio vulgaris* that contains rubredoxin centers and a hemerythrin-like binuclear iron cluster. *Biochemistry* 27:1636-1642.
20. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. Messing, J., and J. Viera. 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. *Gene* 19:269-276.
22. Miller, J. H. 1977. Experiments in molecular genetics, 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. Odom, J. M., and H. D. Peck, Jr. 1984. Hydrogenase, electron transfer proteins, and energy coupling in the sulfate-reducing bacteria *Desulfovibrio*. *Annu. Rev. Microbiol.* 38:551-592.
24. Papavassilia, P., and E. C. Hatchikian. 1985. Isolation and characterization of a rubredoxin and a two-(4Fe-4S) ferredoxin from *Thermodesulfobacterium commune*. *Biochim. Biophys. Acta* 810:1-11.
25. Petitdemange, H., R. Marczak, H. Blusson, and R. Gay. 1979. Isolation and properties of reduced nicotinamide adenine dinucleotide-rubredoxin oxidoreductase of *Clostridium acetobutylicum*. *Biochem. Biophys. Res. Commun.* 91:1258-1265.
26. Pfennig, N., F. Widdel, and H. T. Trüper. 1981. Dissimilatory sulphate-reducing bacteria, p. 931-932. *In* M. P. Starr (ed.), *The prokaryotes*, vol. 1, Springer-Verlag, New York.
27. Postgate, J. R. 1984. The sulphate-reducing bacteria, 2nd ed., p. 1-82. Cambridge University Press, Cambridge.
28. Postgate, J. R., H. M. Kent, and R. L. Robson. 1986. DNA from diazotrophic *Desulfovibrio* strains is homologous to *Klebsiella pneumoniae* structural *nif* DNA and can be chromosomal or plasmid-borne. *FEMS Microbiol. Lett.* 33:159-163.
29. Rosenberg, M., and D. Court. 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. *Annu. Rev. Genet.* 13:319-353.
30. Russo, A. F., and D. E. Koshland, Jr. 1983. Separation of signal transduction and adaption functions of the aspartate receptor in bacterial sensing. *Science* 220:1016-1020.
31. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463-5467.
32. Sieker, L. C., R. E. Stenkamp, L. H. Jensen, B. Prickril, and J. Le Gall. 1986. Structure of rubredoxin from the bacterium *Desulfovibrio desulfuricans*. *FEBS Lett.* 208:73-76.
33. Staden, R. 1984. Graphical methods to determine the function of nucleic acid sequences. *Nucleic Acids Res.* 12:521-538.
34. Staden, R., and A. D. McLachlan. 1982. Codon preference and its use in identifying protein coding regions in long DNA sequences. *Nucleic Acids Res.* 10:141-156.
35. Viera, J., and J. Messing. 1982. The pUC plasmid, an M13mp7 derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene* 19:259-268.
36. Vogel, H., M. Bruschi, and J. Le Gall. 1977. Phylogenetic studies of two rubredoxins from sulfate reducing bacteria. *J. Mol. Evol.* 9:111-119.
37. Voordouw, G. 1988. Cloning of genes encoding redox proteins of known amino acid sequence from a library of the *Desulfovibrio vulgaris* (Hildenborough) genome. *Gene* 69:75-83.
38. Voordouw, G., and S. Brenner. 1985. Nucleotide sequence of the gene encoding the hydrogenase from *Desulfovibrio vulgaris* (Hildenborough). *Eur. J. Biochem.* 148:515-520.
39. Voordouw, G., H. M. Kent, and J. R. Postgate. 1987. Identification of the genes for hydrogenase and cytochrome  $c_3$  in *Desulfovibrio*. *Can. J. Microbiol.* 33:1006-1010.
40. Voordouw, G., J. E. Walker, and S. Brenner. 1985. Cloning of the gene encoding the hydrogenase from *Desulfovibrio vulgaris* (Hildenborough) and the determination of the  $\text{NH}_2$ -terminal sequence. *Eur. J. Biochem.* 148:509-514.
41. Watenpaugh, K. D., T. N. Margulis, L. C. Sieker, and L. H. Jensen. 1978. Water structure in a protein crystal: rubredoxin at 1.2 Å resolution. *J. Mol. Biol.* 122:175-190.
42. Watenpaugh, K. D., L. C. Sieker, and L. H. Jensen. 1979. The structure of rubredoxin at 1.2 Å resolution. *J. Mol. Biol.* 131:509-522.
43. Watenpaugh, K. D., L. C. Sieker, and L. H. Jensen. 1980. Crystallographic refinement of rubredoxin at 1.2 Å resolution. *J. Mol. Biol.* 138:615-633.
44. Wolin, E. A., M. J. Wolin, and R. F. Wolfe. 1963. Formation of methane by bacterial extracts. *J. Biol. Chem.* 238:2882-2886.



## THE CHELATE STRUCTURE OF *MICROCOCCUS AEROGENES* RUBREDOXIN\*

BY HELMUT BACHMAYER,<sup>†</sup> KERRY T. YASUNOBU,<sup>‡</sup> AND H. R. WHITELEY

DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS, UNIVERSITY OF HAWAII, HONOLULU,  
AND DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF WASHINGTON, SEATTLE

Communicated by Albert C. Smith, January 25, 1968

Rubredoxin represents the simplest type of nonheme iron protein, as it is a small molecule (mol wt 6000) containing only 1 gm atom of iron and no "labile sulfur."<sup>1</sup> In addition, it lacks the amino acids histidine and arginine<sup>1, 2</sup> which are potential ligands of iron. A knowledge of the amino acid sequence of *Micrococcus aerogenes* rubredoxin<sup>2</sup> has aided in the study of the chelate structure. Previous investigations have demonstrated that the iron in rubredoxin exists in the form of an octahedral complex in which four of the ligands are cysteine residues.<sup>3</sup> In the present communication, results are presented concerning the nature of the fifth and sixth ligand of the ferric iron.

This paper describes various chemical modifications of native rubredoxin and its apoprotein. The ability of modified apoprotein to reconstitute with ferric iron was followed by absorption and electron paramagnetic resonance (EPR) spectra.

**Materials and Methods.—Preparation of rubredoxin and derivatives:** The procedure for isolation of purified rubredoxin has been previously reported.<sup>3</sup> The preparations used in the present study possessed an  $E_{280\text{m}\mu}/E_{490\text{m}\mu}$  ratio of 2.4. The apoprotein was prepared by precipitation with trichloroacetic acid as previously described.<sup>3</sup> Protein concentrations were determined by amino acid analyses of aliquots of stock solutions after hydrolyzing the samples for 24 hr in 6 *N* HCl.

**Reconstitution of rubredoxin:** The lyophilized apoprotein (0.05–0.1  $\mu$ mole) was dissolved in 0.9 ml of 1 *M* NaCl–0.04 *M* veronal–HCl buffer, pH 7.5. To this solution 0.1 ml of dithiothreitol (aqueous solution, 0.5 *M*) was added and the mixture incubated at 40° for 5 min. Afterward, 0.01 ml of a neutral FeCl<sub>3</sub> solution (0.5 mg Fe/ml) was added, and the mixture maintained at 40° for a further 10 min.

**Reaction with 2-hydroxy-5-nitrobenzylbromide:**<sup>4</sup> Dried aporubredoxin (0.05–0.1  $\mu$ mole) was dissolved in 0.8 ml of acetate buffer (0.17 *M*, pH 4.6) and reacted with a 70-fold molar excess of 2-hydroxy-5-nitrobenzylbromide in acetone. The reaction was allowed to proceed in the dark for 2 hr at 25°. Excess reagent was removed by passing the reaction mixture through a column of Sephadex G-25.

**Dinitrophenylation:** Dinitrophenylation<sup>5</sup> of the native protein (0.022  $\mu$ mole) was carried out in 4% NaHCO<sub>3</sub>, in NaHCO<sub>3</sub>–Na<sub>2</sub>CO<sub>3</sub> buffer, pH 10, or in a 4% NaHCO<sub>3</sub>–50% ethanol mixture using a 2000-fold excess of fluorodinitrobenzene (FDNB) in a total volume of 0.2 ml. Reactions were carried out in the dark for 2 hr at 25° or 60°. The reagent and side products of the reaction were removed by gel filtration on Sephadex G-25.

**Oxidation of tryptophan with *N*-bromosuccinimide (NBS):**<sup>6</sup> The NBS used was recrystallized from water. For the reaction, 0.1  $\mu$ mole of rubredoxin or aporubredoxin was dissolved in 0.9 ml of 8 urea–0.33 *M* acetate buffer, pH 4.0. Increasing amounts of fresh solution (0.015 *M*) of NBS in water were added and the change in absorbance at 280 and 490 m $\mu$  were recorded.

**Acetylation with *N*-acetyl imidazole:**<sup>7</sup> About 0.1  $\mu$ mole of native rubredoxin or aporubredoxin was reacted with a 60-fold excess of *N*-acetyl imidazole (K and K Lab.) in 0.9 ml of 0.05 *M* veronal–HCl, pH 7.5, containing 1 *M* NaCl. The reaction was carried out at 25° for varying periods of time. The acetylated protein was purified on Sephadex

G-25. In the experiments with C-14-labeled reagent (Cal Biochem, sp. act. 22.28 mc/mM), identical conditions were used and after purification on a column of Sephadex G-25, radioactivity was measured in a liquid scintillation counter. The acetyl groups were removed by the addition of 0.1 ml of a 10% solution of hydroxylamine-HCl adjusted to pH 7.5 with NaOH and incubating for 15 min at 25°.

*Reaction with O-methyl isourea:*<sup>3</sup> In a typical experiment, 0.066  $\mu$ mole of aporubredoxin, dissolved in 0.1 ml of 0.2 M Na<sub>2</sub>CO<sub>3</sub>, was reacted with 0.05 ml of 1 M O-methyl isourea (K and K Lab.). In some experiments 95% ethanol was added to the reaction mixture to make the solution 50% with respect to ethanol. The reactions were carried out for 4–7 days at 4°. The excess reagent was removed by passing the reaction mixture through Sephadex G-25. The homoarginine content was determined in acid hydrolysates (24 hr) on the short column of the automatic amino acid analyzer.

*Results.—Experiments with native rubredoxin:* The reaction of chemical reagents with *M. aerogenes* rubredoxin was investigated to determine the possible ligands chelated to iron. The loss in color of the red protein was used to determine whether or not the chelate structure in the native protein was altered by the chemical reagents. Of course, the fact that the reagent may be altering groups involved in maintaining the secondary and tertiary structure and thus indirectly causing an alteration of the chelate structure must be kept in mind.

*Possible chelation of the iron with the -NH<sub>2</sub> groups:* The reaction of rubredoxin with fluorodinitrobenzene under various conditions is summarized in Table 1.

TABLE 1. Reactivity of native rubredoxin with FDNB.

Sample	Reaction conditions*	—Reacted Residues of—	
		Lysine	Tyrosine
A	4% NaHCO <sub>3</sub>	1.10	0.50
B	Carbonate buffer, pH 10	1.62	1.00
C	1% NaHCO <sub>3</sub> , 60°	1.73	2.00
D	1% NaHCO <sub>3</sub> , 50% ethanol	1.60	2.60

\* About 0.022  $\mu$ mole of rubredoxin were used in each case. After the reaction was concluded, the reaction mixture was passed through a column of Sephadex G-25. The DNP-protein was then hydrolyzed with 6 N HCl for 18 hr and the amino acid content was determined with an automatic amino acid analyzer. From the known content of the various amino acids in native rubredoxin, the loss of the residues was assumed to be due to reaction with FDNB.

Since the yellow color of the DNP-protein obscured the visible spectrum of the protein, the EPR spectra of the reaction products were investigated, as previously described,<sup>3</sup> to determine whether or not the chelate structure was altered in the reaction. In experiment B, when both lysine residues and one of the tyrosines had reacted, the EPR spectrum was similar to that of the native protein. However, in experiment D, where the dinitrophenylation was carried out in the presence of 50 per cent ethanol, the EPR spectrum characteristic of the native protein was abolished. The two residues of lysine were again found to have reacted but in this case all three tyrosine residues were converted to the O-DNP-derivative. These experiments ruled out lysine as one of the potential ligands and suggested the possibility that one or both of the tyrosine residues, reacting with FDNB only in the presence of 50 per cent ethanol, might be linked to the iron.

*Possibility of methionine as a ligand:* Treatment of the rubredoxin with cyanogen bromide (CNBr)<sup>9</sup> at pH 3 released 20–30 per cent of the NH<sub>2</sub>-terminal methionine as homoserine. In a separate experiment, after the digestion of the native

rubredoxin with leucine aminopeptidase, 20 per cent of the methionine and 17 per cent of the glutamine were found to be released. Neither the absorption spectrum nor the extinction coefficient of the rubredoxin solutions was altered in any of the above-mentioned experiments. Thus, these experiments ruled out a bond between the iron and the sulfur atom or the  $\alpha$ -NH<sub>2</sub> group of the methionine.

**Possible chelation of the iron with the tryptophan residue:** N-bromosuccinimide readily bleached the spectrum of rubredoxin. The velocity of the reaction depended on the amount of reagent used. In 0.33 M acetate buffer, pH 4, containing 8 M urea, the absorbance at 490 m $\mu$  dropped 75 per cent after three hours of reaction at a NBS-to-rubredoxin ratio of 1:1. At a ratio of 2.2:1, a drop of 96 per cent of the 490 m $\mu$  peak was observed after 2½ hours, as shown in Figure 1.

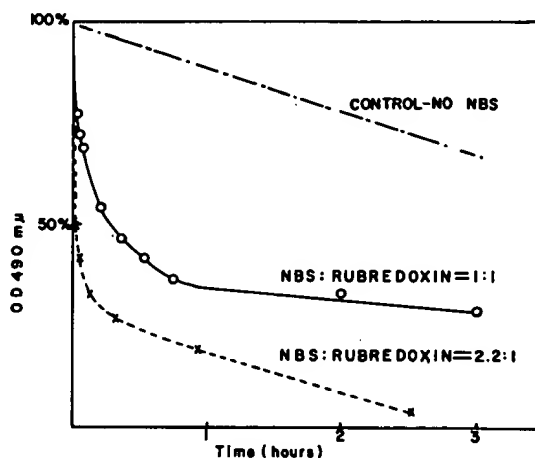


FIG. 1.—Decolorization of rubredoxin with N-bromosuccinimide (NBS). The degree of deterioration was measured by the drop of the OD<sub>490</sub> as a function of reaction time at room temperature for different ratios of reagent to rubredoxin.

According to Funatsu *et al.*,<sup>6</sup> under these conditions, the only residue which should react significantly is tryptophan. The experiments suggested a need for further investigations of the possible role of tryptophan as a potential candidate in iron binding.

**Experiments with aporubredoxin:** Aporubredoxin was readily prepared by incubation with trichloroacetic acid. It was possible to reconstitute it 85–100 per cent by the addition of dithiothreitol and FeCl<sub>3</sub> (see *Materials and Methods*). The fully reconstituted protein had an absorption spectrum and EPR signal identical to that of the native protein. As a consequence of this finding, the reconstitution of modified apoprotein with iron could be investigated as well.

**Acetylation of the tyrosine residues of aporubredoxin:** The hydroxyl groups of the three tyrosine residues in aporubredoxin were acetylated by reaction with *N*-acetyl imidazole. The extent of the reaction was followed by using *N*-acetyl imidazole (acetyl-C-14) or by following the decrease in absorbancy at 280 m $\mu$ . The acetylated derivatives could no longer be reconstituted. Removal of the acetyl groups with hydroxylamine restored the ability of the apoenzyme to recombine with FeCl<sub>3</sub>, as shown in Figure 2.

**Possible involvement of tryptophan:** Aporubredoxin was reacted with NBS in 8 M urea, pH 4.0. From the drop in absorbance at 280 m $\mu$ , about 90 per cent of

the tryptophan residue was converted to the oxindole-derivative. The reaction product was purified on a column of Sephadex G-25 and the reconstitution experiment was attempted but only 10 per cent of the derivative was reconstitutable.

Another sample of aporubredoxin was reacted with 2-hydroxy-5-nitrobenzyl-bromide and the one tryptophan residue was substituted. This derivative could

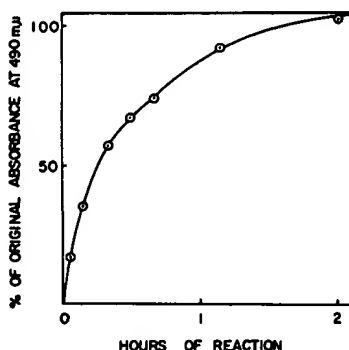


FIG. 2.—Deacetylation of acetyl rubredoxin with hydroxylamine. The reaction mixture contained 0.05  $\mu$ mole aporubredoxin, dissolved in 1.0 ml veronal-HCl, pH 7.5, which contained 1 *M* NaCl. After 30 min of reaction with a 60-fold molar excess of *N*-acetyl imidazole, Cleland's reagent and FeCl<sub>3</sub> were added, as described for the reconstitution experiment in the experimental part. No color developed. Then 0.1 ml of 10% hydroxylamine (pH adjusted to 7.5) was added to the reaction mixture and the development of color followed as a function of time.

not be reconstituted. These experiments implicated tryptophan as a potential ligand of the iron.

*Conversion of lysine to homoarginine:* The two lysine residues of rubredoxin were converted to homoarginine by reacting aporubredoxin with *O*-methyl isourea. About 75–80 per cent of the lysine residues reacted. The derivatives could still be reconstituted to rubredoxin to the same extent as the untreated apoprotein.

*Discussion.*—Two approaches were used to determine the ligands 5 and 6 chelated to iron, in addition to the four cysteine residues, in the octahedral chelate structure present in rubredoxin. The first approach involved modifications of native rubredoxin with various chemical reagents and their influence on the spectral properties. A loss of the characteristic absorption spectrum, especially the maxima at 380 and 490  $m\mu$ , and of the EPR signal were used as criteria for changes of the iron complex. These studies do not necessarily prove that a specific functional group is linked to the iron since the substitution may have altered the secondary and tertiary structure of rubredoxin. Nonetheless, experiments

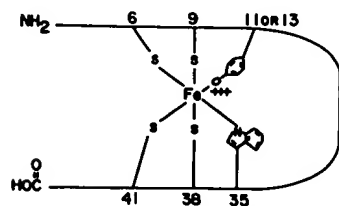


FIG. 3.—Schematic drawings of the possible ligands of rubredoxin. Numbers indicate position of amino acid residues in the peptide chain.

with suitable tyrosine and tryptophan reagents indicated that a tyrosine phenolic group and the indole nitrogen of the sole tryptophan residue are linked to the iron.

A more direct approach was made possible by the preparation of aporubredoxin which could be reactivated by reducing agents plus ferric iron. An important point is the fact that many of the functional groups are shielded in the native rubredoxin. However, the apoprotein reacts readily with most chemical reagents, suggesting a more open confirmation. The ability of the apoprotein

to recombine with ferric iron under suitable conditions is abolished by reagents which are known to modify tyrosine hydroxyl groups and the indole ring of tryptophan. From similar experiments with reagents specifically modifying the  $\epsilon$ -NH<sub>2</sub> group of the lysine residues, this amino acid could definitely be excluded as an iron ligand. Consequently, we propose the chelate structure in Figure 3 with a phenolic hydroxyl group and the nitrogen of the indole ring as fifth and sixth ligand of the iron chelate of *M. aerogenes* rubredoxin.

This view is supported by the homology in the amino acid sequence of two different rubredoxins. In both *M. aerogenes* and *Peptostreptococcus elsdenii* rubredoxin<sup>10</sup> the sole tryptophan residue occupies position 35, and tyrosine residues are found in positions 11 and 13. On the other hand, the tyrosine in position 50 of *M. aerogenes* rubredoxin is replaced by a lysine residue in the *P. elsdenii* protein which makes it unlikely that this tyrosine is bound to the iron.

**Summary.**—The two lysine residues and the NH<sub>2</sub>-terminal methionine are definitely not linked to the iron in the chelate structure of *M. aerogenes* rubredoxin. Evidence is presented indicating that ligands 5 and 6 are the phenolic hydroxyl group of a tyrosine residue (residues 11 or 13) and the indole nitrogen of the tryptophan residue in position 35. The evidence is based on the reaction of group-specific chemical reagents with native rubredoxin and its apoprotein.

\* This work was made possible by grant GB 6608 from the National Science Foundation and grant CA-03031 from the Cancer Institute of the National Institutes of Health.

† Present address: Institute of Molecular Biology, Wasagasse 9, A 1090 Vienna, Austria.

‡ To whom requests for reprints should be addressed.

<sup>1</sup> Lovenberg, W., and B. E. Sobel, these PROCEEDINGS, 54, 193 (1965).

<sup>2</sup> Bachmayer, H., K. T. Yasunobu, and H. R. Whiteley, *Biochem. Biophys. Res. Commun.*, 26, 435 (1967).

<sup>3</sup> Bachmayer, H., L. H. Piette, K. T. Yasunobu, and H. R. Whiteley, these PROCEEDINGS, 57, 122 (1967).

<sup>4</sup> Horton, H. R., and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, 87, 1126 (1965).

<sup>5</sup> Fraenkel-Conrat, H., J. L. Harris, and A. L. Levy, *Methods Biochem. Anal.*, 2, 359 (1955).

<sup>6</sup> Funatsu, M., N. M. Green, and B. Witkop, *J. Am. Chem. Soc.*, 86, 1846 (1964).

<sup>7</sup> Simpson, R. T., J. F. Riordan, and B. L. Vallee, *Biochemistry*, 2, 616 (1963).

<sup>8</sup> Hettinger, T. P., and H. A. Harbury, these PROCEEDINGS, 52, 1469 (1964).

<sup>9</sup> Gross, E., and B. Witkop, *J. Am. Chem. Soc.*, 83, 1510 (1961).

<sup>10</sup> Bachmayer, H., K. T. Yasunobu, J. L. Peel, and S. Mayhew, *J. Biol. Chem.*, in press.

## Characterization of Ferredoxin, Flavodoxin, and Rubredoxin from *Clostridium formicoaceticum* Grown in Media with High and Low Iron Contents

STEVE W. RAGSDALE AND LARS G. LJUNGDAHL\*

Department of Biochemistry, University of Georgia, Athens, Georgia 30602

Received 15 July 1983/Accepted 24 October 1983

Ferredoxin, flavodoxin, and rubredoxin were purified to homogeneity from *Clostridium formicoaceticum* and characterized. Variation of the iron concentration of the growth medium caused substantial changes in the concentrations of ferredoxin and flavodoxin but not of rubredoxin. The ferredoxin has a molecular weight of 6,000 and is a four iron-four sulfur protein with eight cysteine residues. The spectrum is similar to that of other ferredoxins. The molar extinction coefficients are  $22.6 \times 10^3$  and  $17.6 \times 10^3$  at 280 and 390 nm, respectively. From 100 g wet weight of cells grown with 3.6  $\mu$ M iron and with 40  $\mu$ M iron, 5 and 20 mg of ferredoxin were isolated, respectively. The molecular weight of rubredoxin is 5,800 and it contains one iron and four cysteines. The UV-visible absorption spectrum is dissimilar to those of other rubredoxins in that the 373 nm absorption peak is quite symmetric, lacking the characteristic 350-nm shoulder found in other rubredoxins. The flavodoxin is a 14,500-molecular-weight protein which contains 1 mol of flavin mononucleotide per mol of protein. It forms a stable, blue semiquinone upon light irradiation in the presence of EDTA or during enzymatic reduction. When cells were grown in low-iron medium, flavodoxin constituted at least 2% of the soluble cell protein; however, it was not detected in extracts of cells grown in high-iron medium. The rubredoxin and ferredoxin expressed during growth in low-iron and high-iron media are identical as judged by iron, inorganic sulfide, and amino acid analysis, as well as light absorption spectroscopy.

Ferredoxin, flavodoxin, and rubredoxin are low-molecular-weight electron-carrier proteins which are important in anaerobic metabolism. In the metabolism of the acetogenic bacteria, ferredoxin is an electron acceptor for numerous low-potential oxidation-reduction reactions, including pyruvate:ferredoxin oxidoreductase (9, 38, 44), hydrogenase (8; Pezacka and Wood, Arch. Microbiol., in press), NADH:ferredoxin oxidoreductase (38, 44), and CO dehydrogenase (6, 17, 34). Flavodoxin has been shown to substitute for ferredoxin in many redox reactions, but at a slower rate of electron transfer (20, 21). Rubredoxin has been found to substitute for ferredoxin in a few oxidation-reduction reactions, but usually at a very low rate, since the redox potential of rubredoxin is approximately 400 mV higher than that of ferredoxin. Recently, rubredoxin was found to be a superior electron acceptor in the CO dehydrogenase reaction in acetogenic bacteria (33, 34). The properties of rubredoxin have been reviewed (10).

We studied these three electron carriers in *Clostridium formicoaceticum* since flavodoxin has not been isolated from acetogenic bacteria and ferredoxin (12, 44) and rubredoxin (43) have been purified from only one acetogen, *Clostridium thermoaceticum*. Characterization of the electron carriers involved in the acetate biosynthetic pathway is important, since the acetogens carry out a reduction of CO<sub>2</sub> to acetate in a sequence of reactions of four two-electron transfers from some electron donor (CO, H<sub>2</sub>, pyruvate, glucose, etc.) to a pathway involving formate dehydrogenase (42) and tetrahydrofolate enzymes (22).

### MATERIALS AND METHODS

Thioglycolic acid, DEAE-Sephadex A50-120, and Sepharose CL-6B-200 were obtained from Sigma Chemical Co. Ultrapure hydrochloric acid was from Alfa Thiokol/Ventrol.

\* Corresponding author.

Ultrogel AcA chromatography gels were from LKB Instruments and Sephadex gels were from Pharmacia. DEAE-cellulose was from Whatman Ltd.

**Growth of *C. formicoaceticum*.** *C. formicoaceticum* ATCC 23439, described by Andreesen et al. (1), was maintained and grown by using the medium of Moore et al. (29) containing 3.6  $\mu$ M iron as described earlier (2). When high-iron medium was used, the concentration of ferrous ammonium sulfate was 40  $\mu$ M.

**Analytical methods.** Protein concentration was determined by the rose bengal dye-binding assay (11), using ovalbumin as standard, or by the extinction coefficients, which were determined as described below. Amino acid analysis was carried out on the homogenous electron carrier proteins by first dialyzing samples for 36 h against double-distilled water and by hydrolyzing in vacuo in ultrapure 6.0 N HCl for 12, 24, 48, and 72 h at 110°C. Tryptophan was determined by acid hydrolysis in 4.0 and 6.0% thioglycolic acid (26), and cysteine was determined as cysteic acid (16). Norleucine was included as internal standard. The partial specific volume was calculated from the amino acid composition (3). The extinction coefficients were calculated by using amino acid analyses to determine the nanomoles of protein relative to the absorbance at a given wavelength. The rose bengal protein assay was standardized for each protein by amino acid analysis.

Sedimentation equilibrium and velocity experiments were performed with a Beckman model E ultracentrifuge equipped with absorption optics. In velocity experiments, the sedimentation coefficient was determined by the moving boundary method (35), using the schlieren peak to detect the position of the boundary as a function of time. Temperature was maintained at 20°C during the velocity and equilibrium experiments. All samples were run in 50 mM potassium phosphate-0.3 M potassium chloride buffer. The sedimentation coefficient was corrected to the condition of water at

TABLE 1. Properties of ferredoxin, rubredoxin, and flavodoxin from *C. formicoaceticum*

Protein	Mol wt determined by:				Partial specific volume <sup>a</sup>	Stokes radius <sup>b</sup>	Iron per mol of protein <sup>c</sup>
	Gel filtration	Sedimentation equilibrium	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis	Amino acid analysis			
Ferredoxin	5,200 ± 500	6,700 ± 200		6,000 ± 100	0.663	9.5 ± 0.4	4.6 ± 0.4
Rubredoxin	5,600 ± 500	5,900 ± 100		5,800 ± 50	0.715	10.0 ± 0.4	1.2 ± 0.2
Flavodoxin	14,000 ± 1,000	16,500	14,500 ± 500	14,000 ± 1,000	0.731	17.4 ± 0.7	ND

20°C. The molecular weights were calculated according to the Svedberg equation (35), assuming a one-component system.

The Stokes radii and molecular weights were determined by using carbonic anhydrase, cytochrome *c*, ovalbumin, chymotrypsinogen A, and RNase A as standards. The correlation coefficient of the line obtained in the standardization was 0.992.

Acid-labile sulfide was analyzed (32) by using sodium sulfide as the standard. Iron was determined with bathophenanthroline (7) and by plasma emission spectroscopy (19). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was done by the method of Weber et al. (41), and standard alkaline electrophoresis was performed with Tris-glycine (5). Preparative electrophoresis was carried out with an LKB 7900 Uniphor system with pH 8.0 Tris-barbital (14).

Flavodoxin was immobilized to Sepharose CL-6B as described by Mayhew and Strating (28) after cyanogen bromide activation (4).

**Purification of flavodoxin, rubredoxin, and ferredoxin from *C. formicoaceticum* cells.** Figure 1 gives a summary of the purification procedure. This procedure describes the purification from low-iron-grown cells; however, identical steps were used in the purification of rubredoxin and ferredoxin from high-iron-grown cells.

**Crude extract.** Frozen cells (205 g) were suspended in 600 ml of 50 mM potassium phosphate buffer, pH 7.2, and extracted as described previously (2). The suspension was centrifuged in a type 35 Beckman rotor at  $57,000 \times g$  for 90 min. The supernatant (21,000 mg of protein) was used in the purification procedure.

**DE23 cellulose.** The supernatant of the ultracentrifugation step was applied to a DE23 cellulose column (5.5 by 38 cm) which was washed with 50 mM potassium phosphate, pH 7.2. The top layer of this column containing the electron carriers was removed with a spatula and placed on a fresh bed of DE23 cellulose equilibrated with Tris-hydrochloride (0.2 M, pH 7.6). The column was washed with 0.25 M Tris-

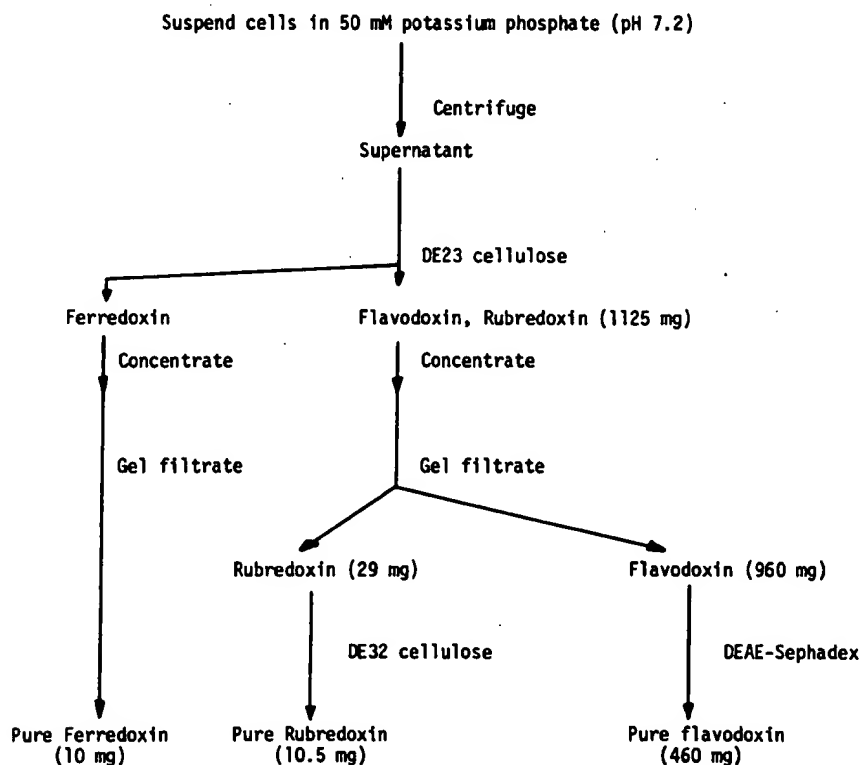


FIG. 1. Purification of rubredoxin, flavodoxin, and ferredoxin from 205 g of *C. formicoaceticum* cells grown in low-iron medium. The final yields of ferredoxin and flavodoxin were dependent upon the concentration of iron in the medium. See the text for details.

TABLE 1. (Continued)

Sulfur per mol of protein	Sedimentation coefficient ( $S_{20,w}$ )	Molar extinction coefficient (liter mol <sup>-1</sup> cm <sup>-1</sup> ) at					
		280 nm	390 nm	490 nm	272 nm	373 nm	444 nm
4.3 ± 0.4	ND <sup>d</sup>	22,600	17,600				
0	ND	18,200		7,300		9,200	
0	2.1	ND			38,600	6,500	7,700

<sup>a</sup> Determined from amino acid analysis.<sup>b</sup> Determined from gel filtration.<sup>c</sup> Average of iron determinations by the Doeg-Ziegler method and by plasma emission spectroscopy, using the amino acid analysis for the determination of the protein concentration.<sup>d</sup> ND, Not determined.

hydrochloride extensively before the flavodoxin and rubredoxin were eluted together with 0.3 M Tris-hydrochloride. The 2-liter eluate was concentrated to 50 ml by using a DE32 cellulose column (2.6 by 20 cm) and then to 15 ml with an Amicon UM-2 ultrafiltration membrane. This concentrate contained 1,125 mg of protein when cells grown in low-iron medium were used. Ferredoxin was eluted from the first DE23 column after the elution of flavodoxin and rubredoxin with 0.5 M Tris-hydrochloride.

**Tandem gel filtration.** The rubredoxin-flavodoxin concentrate from the DE23 step was applied to a tandem Aca 54-Aca 44 column setup (both 2.6 by 85 cm). When run in low-salt buffers, flavodoxin seemed to aggregate, so 0.2 M KCl was included in the 50 mM Tris buffer. Under these conditions, flavodoxin eluted in a tight band. The flavodoxin solution contained 960 mg of protein, whereas the rubredoxin eluate had 29 mg of protein. Rubredoxin eluted well-separated from flavodoxin.

The ferredoxin from DE32 was applied to the same gel filtration setup and run in the same buffer system. The resulting ferredoxin was pure as judged by the absorption ratio (absorbance at 390 nm [ $A_{390}$ ]/ $A_{280}$ ), and by sedimentation velocity and equilibrium experiments.

**DE32 cellulose.** Rubredoxin from the tandem column was adsorbed to a 5-ml DE32 cellulose column equilibrated with Tris-hydrochloride (0.1 M), and a 200-ml linear gradient from 0.2 to 0.5 M Tris-hydrochloride was run. The red, rubredoxin-containing fraction was concentrated on a small DE32 cellulose column from 75 to 2.5 ml. The yield of rubredoxin was 10.5 mg with an absorption ratio ( $A_{280}/A_{490}$ ) between 2.35 and 2.50.

The rubredoxin from this step was judged to be pure by sodium dodecyl sulfate and alkaline electrophoresis, sedimentation velocity, and absorbance ratio. In sedimentation equilibrium experiments the  $\ln$  of the concentration versus  $r^2$  plot was linear, and the molecular weights calculated with both 490- and 280-nm scans were equivalent, which also indicates homogeneity.

**DEAE-Sephadex chromatography and preparative electrophoresis.** Final purification of flavodoxin could be accomplished by either DEAE-Sephadex chromatography or preparative electrophoresis. Flavodoxin from the tandem column was adsorbed to a DEAE-Sephadex column (2.6 by 22 cm) in 50 mM Tris-hydrochloride (pH 7.6) and eluted with a 2-liter gradient of 0 to 0.25 M sodium chloride in 0.1 M Tris-hydrochloride. The yield of flavodoxin was 460 mg with an absorption ratio ( $A_{272}/A_{373}:A_{444}/A_{373}$ ) of 6.36:1.28. Preparative electrophoresis, described above, yielded a protein

with the same characteristics as that obtained in the DEAE-Sephadex step. It was essential to use the pH 8.0 Tris-barbital system in preparative electrophoresis since the Tris-glycine pH 8.9 system caused loss of the flavodoxin cofactor, flavin mononucleotide. Dissociation of flavin mononucleotide from the protein is reversible, however, as shown below. The protein from this step was homogenous as judged by alkaline and sodium dodecyl sulfate electrophoresis and sedimentation equilibrium and velocity experiments.

## RESULTS

**Effect of iron on the relative amounts of electron carriers.** When grown in medium containing only 3.6  $\mu$ M iron salts, flavodoxin is a major component of the soluble cell protein. Approximately 2% of the soluble cell protein (230 mg per 100 g wet weight of cells) was recovered as homogenous flavodoxin. Under these iron-poor conditions, approximately 5 mg of ferredoxin could be recovered per 100 g wet weight of cells. When the medium was supplemented with 40  $\mu$ M of iron, the amount of ferredoxin that could be recovered as the homogenous protein increased four- to fivefold. Flavodoxin was undetectable in extracts of high iron-grown cells. Rubredoxin amounted to 3 to 5 mg per 100 g of cells, and the amount was unaffected by addition of supplemental iron to the growth medium.

**Properties of electron transfer proteins from *C. formicoacetatum*.** The properties of the electron transfer proteins are summarized in Table 1. The amino acid compositions, iron and inorganic sulfur contents, and molecular weights of ferredoxin, rubredoxin, and flavodoxin were the same regardless of whether the cells were grown in high- or low-iron medium. Only one type of ferredoxin or rubredoxin was seen during chromatography in the purification procedures for high- or low-iron-derived proteins, which is in contrast to results with *C. thermoacetatum*, which contains two ferredoxins (12, 44) and two rubredoxins (43).

**Ferredoxin.** The average molecular weight of ferredoxin, calculated from gel filtration, amino acid analysis, and sedimentation equilibrium, was 6,000. Amino acid analysis (Table 2) gave eight cysteines and a partial specific volume of 0.663 ml/g. By both plasma emission spectroscopy and the method of Doeg and Ziegler (7), approximately four irons were found per mole of protein. Four acid-labile sulfides were found per mole protein. The molar extinction coefficients were 22,600 at 280 nm and 17,600 at 390 nm. It was essential to determine protein concentration by using amino acid analysis including an internal standard. Use of the rose



TABLE 2. Amino acid analysis of ferredoxin, rubredoxin, and flavodoxin from *C. formicoaceticum*

Amino acid	Ferredoxin	Rubredoxin	Flavodoxin
Asx	8	8	7
Thr	2	2	4
Ser	2	1	3
Glx	5	5	28
Pro	4	6	4
Gly	4	6	21
Ala	8	3	9
Cys	8	4	1
Val	5	6	8
Met	1	1	9
Ile	4	2	3
Leu	0	0	14
Tyr	2	3	2
Phe	0	2	2
His	0	0	0
Lys	1	3	8
Arg	0	0	2
Trp	0	1	1

bengal (11) or Lowry et al. (24) assay gave values for protein concentration that were seriously in error. The purified ferredoxin had an absorbance ratio ( $A_{390}/A_{280}$ ) of between 0.74 to 0.78 and a spectrum similar to those of other ferredoxins (30).

**Rubredoxin.** The average molecular weight of rubredoxin was 5,800. As in other rubredoxins, four cysteines were found. The number of irons per mole was approximately 1 to 1.5; however, since the extinction coefficients, absorption ratio ( $A_{280}/A_{490}$ ), and cysteine content were so similar to those of the one-iron rubredoxin, we feel that nonessential iron must be absorbed to the protein. Plasma emission spectroscopy revealed the presence of 3.0 Ca, 8.6 K, 4.2 Na, and 2.0 P per mol protein, so it is reasonable that nonfunctional iron could also be bound. By absorbing the purified rubredoxin to DEAE-cellulose and then washing with pH 5.5 sodium citrate buffer (0.1 M), lower iron readings (approximately 1.0 mol of Fe per mol of protein) than those found in the nontreated sample were obtained.

The spectrum of rubredoxin was unlike that of other rubredoxins (Fig. 2) in that the 373-nm peak was quite symmetric. Thus, the *C. formicoaceticum* rubredoxin lacks a shoulder at 350 nm that is characteristic of other rubredoxins (10, 23, 43). The same spectrum was obtained from rubredoxins isolated from both high- and low-iron-derived cells.

Rubredoxin from *C. formicoaceticum* contains no histidine or arginine (Table 2), a common characteristic of rubredoxins. Tryptophan also is present in this rubredoxin. The partial specific volume is 0.715 ml/g.

**Flavodoxin.** The average molecular weight of flavodoxin is 14,500. Extinction coefficients are shown in Table 1. A partial specific volume of 0.730 ml/g was obtained from the amino acid composition (Table 2). The spectrum (Fig. 3) of the stable semiquinone formed by treatment with light in the presence of EDTA (25) or CO plus CO dehydrogenase was very similar to that of the *Megasphaera elsdenii* flavodoxin (27). The cofactor was identified as flavin mononucleotide by coupling the protein to a Sepharose-CL-6B and eluting cofactor with 5% trichloroacetic acid plus 0.3 mM EDTA as described by Mayhew et al. (28). Flavin adenine dinucleotide, flavin mononucleotide, and riboflavin were equilibrated with the column, but only flavin mononucleotide would bind to the Sepharose-bound apoprotein.

## DISCUSSION

The concentration of iron in the growth medium significantly affects the relative concentrations of ferredoxin and flavodoxin, but not of rubredoxin, in cells of *C. formicoaceticum*. Similar findings have been reported for *M. elsdenii* (27) and *Clostridium pasteurianum* (20). With *C. pasteurianum*, the maximal amount of ferredoxin is formed by bacteria grown in media containing 40  $\mu$ M of iron salts (20). From 100 g of *C. formicoaceticum* cells grown in 40  $\mu$ M iron medium we isolated over 20 mg of ferredoxin but found no evidence for a flavodoxin. In contrast, from the same amount of cells grown with 3.6  $\mu$ M iron in the medium, only about 5 mg of ferredoxin was isolated, whereas, flavodoxin constituted almost 2% of the soluble protein.

It is apparent that ferredoxin is replaced by flavodoxin in cells grown in low-iron media. In addition, Schönheit et al. (36) have shown that degradation of ferredoxin occurs in *C. pasteurianum* during periods of iron deprivation and it is utilized as a source of iron. Therefore, it is interesting that under low-iron conditions *C. formicoaceticum* and *M. elsdenii* (27) produce significant amounts of rubredoxin. This may indicate an important role for rubredoxin in these bacteria. However, the only postulated role so far for rubredoxin in anaerobic bacteria involves CO dehydrogenase, since rubredoxin is the most active acceptor of CO dehydrogenase electrons in the acetogenic bacteria (33, 34).

The molecular weights and other properties of ferredoxin, flavodoxin, and rubredoxin from *C. formicoaceticum* were similar to those reported for those proteins from other bacteria. By using amino acid analysis to determine the amount of protein and plasma emission spectroscopy as well as a colorimetric method to determine iron, we found that ferredoxin contained only four Fe and four S per mole of protein. The extinction coefficient, using the same absolute method for determination of protein concentration, agreed with the conclusion that the *C. formicoaceticum* ferredoxin

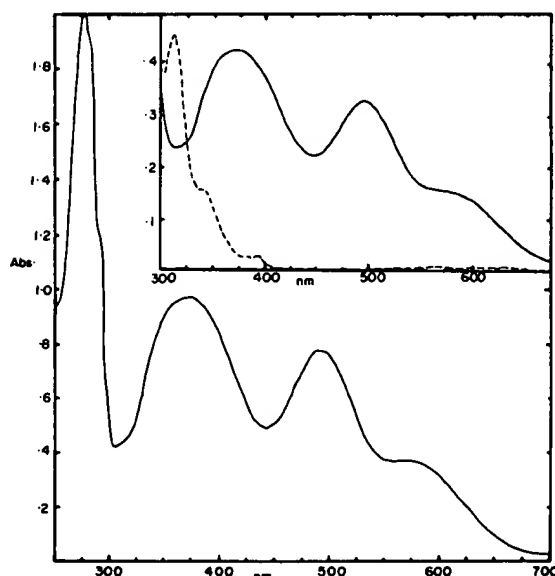


FIG. 2. Light absorption spectrum of *C. formicoaceticum* rubredoxin, 0.64 mg/ml in 50 mM Tris-hydrochloride, pH 7.6. Inset shows rubredoxin, 0.25 mg/ml, oxidized (—) and enzymatically reduced with *C. thermoaceticum* CO dehydrogenase (---) by bubbling with CO for 5 min before addition of enzyme.

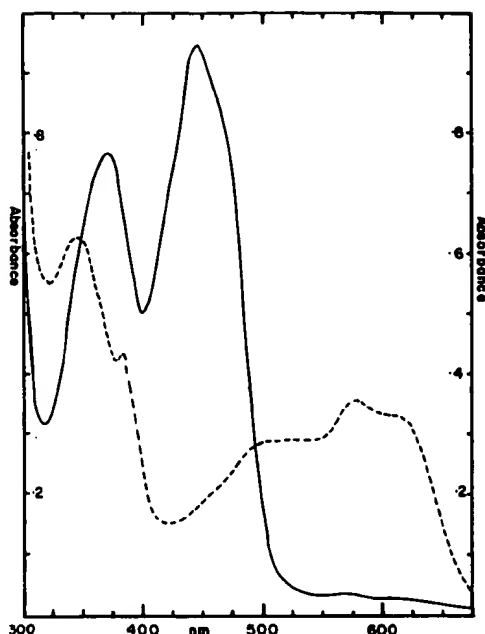


FIG. 3. Light absorption spectrum of *C. formicoaceticum* flavodoxin, 1.80 mg/ml, in 50 mM Tris-hydrochloride, pH 7.6. Oxidized flavodoxin (—) was enzymatically reduced with *C. thermoaceticum* CO dehydrogenase (---) by bubbling with CO for 5 min before addition of enzyme.

is a four-iron ferredoxin, like that of *C. thermoaceticum* ferredoxin I (44) and ferredoxins from *Desulfovibrio gigas* (39), *Desulfovibrio desulfuricans* (45), *Bacillus stearothermophilus* (31), *Spirochaeta stenostrepa* (18), and *Bacillus polymyxa* (37). However, the *C. formicoaceticum* ferredoxin contains eight cysteine residues, whereas *B. stearothermophilus* (15), *Spirochaeta stenostrepa* (18), and *B. polymyxa* (37) ferredoxins contain four and *C. thermoaceticum* (13), *D. gigas* (39), and *D. desulfuricans* (45) ferredoxins have six cysteine residues. Since all ferredoxins so far isolated which contain eight cysteines also contain eight Fe and eight S, it seems that the *C. formicoaceticum* ferredoxin should be convertible into an eight Fe-eight S protein. Even though the purification procedure utilized in preparation of the ferredoxin was quick and rather mild, it is possible that a cluster could have dissociated from the protein. Sequence analysis should explain the difference between this and other eight cysteine-containing ferredoxins.

The *C. formicoaceticum* rubredoxin light absorption peaks and the ratio of the peak at 490 nm to that at 280 nm were similar to those found for other purified rubredoxins. However, the *C. formicoaceticum* we found lacked a shoulder at approximately 350 nm that has been characteristic of other rubredoxins (10, 23, 43). In addition, the electron paramagnetic resonance spectrum (not shown here) of the *C. formicoaceticum* rubredoxin showed only the  $g = 4.3$  resonance, and we did not detect the  $g = 9.4$  resonance seen in other rubredoxins. This  $g = 9.4$  signal is quite temperature sensitive and is always weaker than the  $g = 4.3$  resonance, however, and electron paramagnetic resonance studies at various temperatures have not yet been carried out. We have not yet found the reasons for these spectral differences, since the iron and amino acid analyses were so similar to those of other rubredoxins.

Flavodoxin from *C. formicoaceticum* was similar to other flavodoxins (21, 27). When cells were grown in a medium containing 3.6  $\mu$ M iron salts, it was a major component of the cell and constituted at least 2% of the soluble cell protein. The flavodoxin from *C. formicoaceticum* was used in resonance coherent and anti-Stokes Raman scattering spectroscopy and was found to resemble the *M. elsdenii* flavodoxin, in contrast to the *Desulfovibrio* and *Azotobacter vinelandii* flavodoxins (40).

Recently, ferredoxin, rubredoxin, and flavodoxin from *C. formicoaceticum* have been used as electron acceptors for *C. thermoaceticum* (33), *C. formicoaceticum*, and *Acetobacterium woodii* carbon monoxide dehydrogenases (34). We found in all cases that rubredoxin is the best natural electron acceptor, followed by ferredoxin and the flavodoxin. We postulate that rubredoxin, therefore, is the primary natural electron carrier for CO dehydrogenase in acetogenic bacteria.

#### ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AM-27323 from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases and by project DE-AS09-79 ER10499 from the U.S. Department of Energy at the University of Georgia. S.R. is the recipient of a University of Georgia University-wide graduate assistantship.

We are grateful to Joan E. Clark (University of Georgia) for preparation of the *C. formicoaceticum* cells, Rick Thomas and Ronald Makula for large-scale cell growth, and Dan V. DerVartanian and Susan Holmes (University of Georgia) for carrying out electron paramagnetic resonance experiments.

#### LITERATURE CITED

- Andreesen, J. R., G. Gottschalk, and H. G. Schlegel. 1970. *Clostridium formicoaceticum* nov. spec. Isolation, description and distinction from *Clostridium acetatum* and *Clostridium thermoaceticum*. Arch. Mikrobiol. 72:154-174.
- Clark, J. E., and L. G. Ljungdahl. 1982. Purification and properties of 5,10-methylenetetrahydrofolate cyclohydrolase from *Clostridium formicoaceticum*. J. Biol. Chem. 257:3833-3836.
- Cohn, E. J., and J. T. Edsall. 1943. Density and apparent specific volume of proteins, p. 370-381. In E. J. Cohn and J. T. Edsall (ed.), Proteins, amino acids and peptides as ions and dipolar ions. Reinhold, New York.
- Cuatrecasas, P. 1970. Protein purification by affinity chromatography. J. Biol. Chem. 245:3059-3065.
- Davis, B. J. 1964. Disc electrophoresis-II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121:404-427.
- Diekert, G. B., and R. K. Thauer. 1978. Carbon monoxide oxidation by *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. J. Bacteriol. 136:597-606.
- Doeg, K. A., and D. M. Ziegler. 1962. Simplified methods for the estimation of iron in mitochondria and submitochondrial fractions. Arch. Biochem. Biophys. 97:37-40.
- Drake, H. L. 1982. Demonstration of hydrogenase in extracts of the homoacetate-fermenting bacterium *Clostridium thermoaceticum*. J. Bacteriol. 150:702-709.
- Drake, H. L., S.-I. Hu, and H. G. Wood. 1981. Purification of five components from *Clostridium thermoaceticum* which catalyze synthesis of acetate from pyruvate and methyltetrahydrofolate. J. Biol. Chem. 256:11137-11144.
- Eaton, W. A., and W. Lovenberg. 1973. The iron-sulfur complex in rubredoxin, p. 131-162. In W. Lovenberg (ed.), Iron-sulfur proteins, vol. 2. Academic Press, Inc., New York.
- Elliott, J. I., and J. M. Brewer. 1978. The inactivation of yeast enolase by 2,3-butanedione. Arch. Biochem. Biophys. 190:351-357.

12. Elliott, J. I., and L. G. Ljungdahl. 1982. Isolation and characterization of an  $\text{Fe}_4\text{S}_4$  ferredoxin (ferredoxin II) from *Clostridium thermoaceticum*. *J. Bacteriol.* 151:328-333.
13. Elliott, J. I., S.-S. Yang, L. G. Ljungdahl, J. Travis, and C. F. Reilly. 1982. Complete amino acid sequence of the 4Fe-4S, thermostable ferredoxin from *Clostridium thermoaceticum*. *Biochemistry* 21:3294-3298.
14. Gabriel, O. 1971. Analytical disc gel electrophoresis. *Methods Enzymol.* 22:565-578.
15. Hase, T., N. Ohmiya, H. Matsubara, R. N. Mullinger, K. K. Rao, and D. O. Hall. 1976. Amino acid sequence of a four-iron-four sulfur ferredoxin isolated from *Bacillus stearothermophilus*. *Biochem. J.* 159:55-63.
16. Hirs, C. H. W. 1967. Determination of cystine as cysteic acid. *Methods Enzymol.* 11:59-62.
17. Hu, S.-I., H. L. Drake, and H. G. Wood. 1982. Synthesis of acetyl coenzyme A from carbon monoxide, methyltetrahydrofolate, and coenzyme A by enzymes from *Clostridium thermoaceticum*. *J. Bacteriol.* 149:440-448.
18. Johnson, P. W., E. Canale-Parola. 1973. Properties of rubredoxin and ferredoxin isolated from *Spirochetes*. *Arch. Mikrobiol.* 89:341-353.
19. Jones, J. B., Jr. 1977. Elemental analysis of soil extracts and plant tissue ash by plasma emission spectroscopy. *Commun. Soil Sci. Plant Anal.* 8:349-365.
20. Knight, E., Jr. and R. W. F. Hardy. 1966. Isolation and characteristics of flavodoxin from nitrogen-fixing *Clostridium pasteurianum*. *J. Biol. Chem.* 241:2752-2756.
21. Knight, E., Jr., and R. W. F. Hardy. 1967. Flavodoxin. Chemical and biological properties. *J. Biol. Chem.* 242:1370-1374.
22. Ljungdahl, L. G., and H. G. Wood. 1982. Acetate biosynthesis, p. 165-202. *In* D. Dolphin (ed.), B<sub>12</sub>, vol. 2. John Wiley & Sons, Inc., New York.
23. Lovenberg, W., and B. E. Sobel. 1965. Rubredoxin: a new electron transfer protein from *Clostridium pasteurianum*. *Proc. Natl. Acad. Sci. U.S.A.* 54:193-199.
24. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
25. Massey, V., and G. Palmer. 1966. On the existence of spectrally distinct classes of flavoprotein semiquinones. A new method for the quantitative production of flavoprotein semiquinones. *Biochemistry* 5:3181-3189.
26. Matsubara, H., and R. M. Sasaki. 1969. High recovery of tryptophan from the acid hydrolysates of proteins. *Biochem. Biophys. Res. Commun.* 35:175-181.
27. Mayhew, S. G., and V. Massey. 1969. Purification and characterization of flavodoxin from *Peptostreptococcus elsdenii*. *J. Biol. Chem.* 244:794-802.
28. Mayhew, S. G., and M. J. J. Strating. 1975. Properties of immobilized flavodoxin from *Peptostreptococcus elsdenii*. *Eur. J. Biochem.* 59:539-544.
29. Moore, M. R., W. E. O'Brien, and L. G. Ljungdahl. 1974. Purification and characterization of nicotinamide adenine dinucleotide-dependent methylenetetrahydrofolate dehydrogenase from *Clostridium formicoaceticum*. *J. Biol. Chem.* 249:5250-5253.
30. Mortenson, L. E., and G. Nakos. 1973. Bacterial ferredoxins and/or iron-sulfur proteins as electron carriers, p. 37-64. *In* W. Lovenberg (ed.), *Iron-sulfur proteins*, vol. 1. Academic Press, Inc. New York.
31. Mullinger, R. N., R. Cammack, K. K. Rao, D. O. Hall, D. P. E. Dickson, C. E. Johnson, J. D. Rush, and A. Simopoulos. 1975. Physicochemical characterization of the four-iron four-sulfide ferredoxin from *Bacillus stearothermophilus*. *Biochem. J.* 151:75-83.
32. Rabinowitz, J. C. 1978. Analysis of acid-labile sulfide and sulfhydryl groups. *Methods Enzymol.* 53:275-277.
33. Ragsdale, S. W., J. E. Clark, L. G. Ljungdahl, L. L. Lundie, and H. L. Drake. 1983. Properties of purified carbon monoxide dehydrogenase from *Clostridium thermoaceticum*, a nickel, iron-sulfur protein. *J. Biol. Chem.* 258:2364-2369.
34. Ragsdale, S. W., L. G. Ljungdahl, and D. V. DerVartanian. 1983. Isolation of the carbon monoxide dehydrogenase from *Acetobacterium woodii* and comparison of its properties with the *Clostridium thermoaceticum* enzyme. *J. Bacteriol.* 155:1224-1237.
35. Schachman, H. K. 1959. Ultracentrifugation in biochemistry. Academic Press, Inc., New York.
36. Schönheit, P., A. Brandis, and R. K. Thauer. 1979. Ferredoxin degradation in growing *Clostridium pasteurianum* during periods of iron deprivation. *Arch. Microbiol.* 120:73-76.
37. Stombaugh, N. A., R. H. Burris, and W. H. Orme-Johnson. 1973. Ferredoxins from *Bacillus polymyxa*. Low potential iron-sulfur protein which appear to contain four iron, four sulfur centers accepting a single electron on reduction. *J. Biol. Chem.* 248:7951-7956.
38. Thauer, R. K. 1972.  $\text{CO}_2$  reduction to formate by NADPH. The initial step in the total synthesis of acetate from  $\text{CO}_2$  in *Clostridium thermoaceticum*. *FEBS Lett.* 27:111-115.
39. Travis, J., D. J. Newman, J. LeGall, and H. D. Peck, Jr. 1971. The amino acid sequence of ferredoxin from the sulfate reducing bacterium, *Desulfovibrio gigas*. *Biochem. Biophys. Res. Commun.* 45:452-458.
40. Visser, A. J. W. G., J. Vervoort, D. J. O'Kane, J. Lee, and L. A. Carreira. 1983. Raman spectra of flavin bound in flavodoxins and in other flavoproteins. *Eur. J. Biochem.* 131:639-645.
41. Weber, K., J. R. Pringle, and M. Osborn. 1972. Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. *Methods Enzymol.* 26:3-27.
42. Yamamoto, I., T. Saiki, S.-M. Liu, and L. G. Ljungdahl. 1983. Purification and properties of NADP-dependent formate dehydrogenase from *Clostridium thermoaceticum*, a tungsten-selenium-iron protein. *J. Biol. Chem.* 258:1826-1832.
43. Yang, S.-S., L. G. Ljungdahl, D. V. DerVartanian, and G. D. Watt. 1980. Isolation and characterization of two rubredoxins from *Clostridium thermoaceticum*. *Biochim. Biophys. Acta* 590:24-33.
44. Yang, S.-S., L. G. Ljungdahl, and J. LeGall. 1977. A four-iron, four-sulfide ferredoxin with high thermostability from *Clostridium thermoaceticum*. *J. Bacteriol.* 130:1084-1090.
45. Zubieta, J. A., R. Mason, and J. R. Postgate. 1973. A four-iron ferredoxin from *Desulfovibrio desulfuricans*. *Biochem. J.* 133:851-854.

## Purification and Characterization of Cytochrome $c_3$ , Ferredoxin, and Rubredoxin Isolated from *Desulfovibrio* *desulfuricans* Norway

MIREILLE BRUSCHI,\* CLAUDE E. HATCHIKIAN, LUDMILLA A. GOLOVLEVA,<sup>1</sup> AND  
JEAN LE GALL

Laboratoire de Chimie Bactérienne, Centre National de la Recherche Scientifique, 13274 Marseille  
Cedex 2, France

Received for publication 5 August 1976

Different electron carriers of the non-desulfoviridin-containing, sulfate-reducing bacterium *Desulfovibrio desulfuricans* (Norway strain) have been studied. Two nonheme iron proteins, ferredoxin and rubredoxin, have been purified. This ferredoxin contains four atoms of non-heme iron and acid-labile sulfur and six residues of cysteine per molecule. Its amino acid composition suggests that it is homologous with the other *Desulfovibrio* ferredoxins. The rubredoxin is also an acidic protein of 6,000 molecular weight and contains one atom of iron and four cysteine residues per molecule. The amino acid composition and molecular weight of the cytochrome  $c_3$  from *D. desulfuricans* (strain Norway 4) are reported. Its spectral properties are very similar to those of the other cytochromes  $c_3$  (molecular weight, 13,000) of *Desulfovibrio* and show that it contains four hemes per molecule. This cytochrome has a very low redox potential and acts as a carrier in the coupling of hydrogenase and thiosulfate reductase in extracts of *Desulfovibrio gigas* and *Desulfovibrio desulfuricans* (Norway strain) in contrast to *D. gigas* cytochrome  $c_3$  (molecular weight, 13,000). A comparison of the activities of the cytochrome  $c_3$  (molecular weight, 13,000) of *D. gigas* and that of *D. desulfuricans* in this reaction suggests that these homologous proteins can have different specificity in the electron transfer chain of these bacteria.

The classification of sulfate-reducing bacteria within the genus *Desulfovibrio* is based upon several properties: absence of sporulation, desoxyribonucleic acid composition, growth on certain carbon sources, and, particularly, presence of desulfoviridin and cytochrome  $c_3$  (molecular weight [MW], 13,000) (22).

The Norway strain of *Desulfovibrio desulfuricans* (19), although in most respects typically a *Desulfovibrio*, is characterized by an absence of desulfoviridin. It has recently been demonstrated by Jin-Po Lee et al. (14) that another pigment, desulforubidin, replaces desulfoviridin as a sulfite reductase for this organism. It was thus of taxonomic and biochemical interest to investigate the electron carrier content of the Norway strain to see if other differences could be found between the latter and other strains of *Desulfovibrio* such as *D. vulgaris*, strain Hiltenborough, *D. gigas*, and *D. desulfuricans* strain E1 Agheila Z, all known to contain desulfoviridin as a sulfite reductase.

<sup>1</sup> Present address: Institute of Biochemistry and Physiology of Microorganisms, Academy of Sciences of U.S.S.R., Moscow, U.S.S.R.

In this work we report the purification from the Norway strain of several electron carrier proteins, together with some of their physicochemical properties and their reactivity toward thiosulfate reduction.

### MATERIALS AND METHODS

**Organism and growth conditions.** *D. desulfuricans* (strain Norway 4, National Collection of Industrial Bacteria no. 8310) was grown in a lactate-sulfate medium and harvested as published previously (16).

**Enzyme assays.** A manometric assay was utilized for determination of the biological activity of the purified electron carriers utilizing the reduction of thiosulfate with  $H_2$ . The main compartment of each manometric vessel contained 0.1 M phosphate buffer (pH 7.0), the carrier, hydrogenase, and the reductase preparation in a final volume of 3.0 ml. A 15- $\mu$ mol amount of sodium thiosulfate, freshly prepared, was added from a side arm after preincubation of the flask for 30 min under  $H_2$  at 37°C. The center well contained 0.1 ml of 10%  $CdCl_2$  and 0.1 ml of 10 N NaOH. Thiosulfate reductase activity was measured by following the initial rates of hydrogen utilization. The thiosulfate reductase-hydrogenase-

containing extracts, devoid of electron carriers, were prepared from *D. gigas* and *D. desulfuricans* (Norway strain) as already reported (11).

However, as the hydrogenase activity of *D. desulfuricans* appeared to be very sensitive to ammonium sulfate fractionation, pure hydrogenase prepared from *D. gigas* by the method of Bell (G.R. Bell, Ph.D. thesis, University of Georgia, Athens, 1973) and exhibiting a specific activity of 30  $\mu\text{mol}$  of  $\text{H}_2$  consumed/min per mg was added in all cases to the systems to insure an excess of this activity. Ferredoxin and cytochromes  $c_3$  (MW 13,000) and  $c_3$  (MW 28,000) from *D. gigas*, used to study the electron transfer between hydrogenase and thiosulfate reductase, were purified as described previously (4, 15, 16).

**Amino acid analysis.** Protein samples were hydrolyzed for 18, 20, and 48 h at  $110^\circ\text{C}$  in 6.0 M HCl in evacuated, sealed tubes according to the method of Moore and Stein (20). The average was calculated from at least five analyses. The amino acid composition was determined with a Beckman Multichrom amino acid analyzer. The values for serine and threonine have been corrected after extrapolation to zero hydrolysis time. Cysteine and methionine were analyzed after performic acid oxidation as cysteic acid and methionine sulfone, respectively, according to Hirs (12).

**Molar extinction coefficients.** The molar extinction coefficients of the proteins were obtained by measuring the values of the optical densities of their absorption maxima of a solution of known protein concentration. The molarity of the used solutions was calculated from amino acid analysis.

**Iron determination.** Non-heme (ferrous iron) was determined by the o-phenanthroline method of Harvey et al. (10), as modified by Lovenberg et al. (17). The absorption of the complex of the ferrous iron and o-phenanthroline was measured at 512 nm.

**Inorganic sulfide determination.** The inorganic sulfide content of ferredoxin was determined by using an adaptation of the method of Fogo and Popowski (8) by Lovenberg et al. (17). A 1.3-ml amount of 1% zinc acetate and 0.05 ml of 12% sodium hydroxide were added to the sample to be analyzed. A 0.25-ml portion of 0.5% *N,N*-dimethylenephenylenediamine hydrochloride and 0.05 ml of 0.023 M ferric chloride were then added to each tube. After 20 min, 0.85 ml of water was added, and the absorbance was determined at 670 nm ( $A_{670}$ ). The tubes were stoppered and open only for the time necessary for each addition of reagents.

**Molecular weight determination.** The molecular weights were estimated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, using the procedure of Weber and Osborn (27), and by molecular filtration on a Sephadex column.

**Spectral studies.** The visible and ultraviolet spectra were determined with a Cary 14 spectrophotometer. A spectrophotometric method was also used for the monitoring of the purification of the electron carrier proteins, using a specific absorption band: 552 nm for cytochrome  $c_3$ , 390 nm for ferredoxin, and 490 nm for rubredoxin.

## RESULTS

**Protein purification. (i) Preparation of extracts.** In these protein fractionation procedures, unless otherwise noted, all buffers were at pH 7.6 and all operations were performed at  $+4^\circ\text{C}$ .

For preparation of the crude extract, 50 mM tris(hydroxymethyl)aminomethane (Tris)hydrochloride was added to 1,069 g (wet weight) of bacterial paste to bring the volume to 1,600 ml. After addition of a few deoxyribonuclease crystals, the cell suspension was passed twice through a Gaulin homogenizer. The resulting extract was centrifuged for 2 h at  $39,000 \times g$ , and the pellet was discarded.

**(ii) Cytochromes.** The clear extract was stirred overnight with 200 ml of silica gel, and the gel, to which the cytochromes were adsorbed, was separated by decantation. The decanted extract was saved for acidic protein purification (see below). The silica gel was washed several times with 10 volumes of 10 mM Tris-hydrochloride buffer until the supernatant was free of protein. The cytochromes were then eluted from the gel with 1 M  $\text{K}_2\text{HPO}_4$ , giving 650 ml of extract, which was dialyzed overnight against 10 liters of distilled water. The cytochromes were then adsorbed on a calcinated alumina column (4 by 14 cm) equilibrated with 10 mM Tris-hydrochloride buffer. The column was washed with 100 ml of the same buffer, and the protein was eluted with 1 M potassium phosphate buffer, giving 110 ml of extract.

After dialysis against 10 liters of distilled water, the cytochrome fraction was lyophilized, resuspended in 50 ml of 10 mM Tris-hydrochloride buffer, and placed on a Sephadex G-75 column (4 by 200 cm). Three cytochrome bands were separated: the first (with the largest molecular weight) was not purified further; the second contained cytochrome  $c_3$ ; and the third (with the smallest molecular weight) contained a cytochrome presenting a "split  $\alpha$ " band upon reduction with ascorbic acid. This cytochrome was called cytochrome  $c_{355/350}$  by analogy with the cytochrome found by Shioi et al. (24) in *Chloropseudomonas ethylica*.

**(iii) Purification of cytochrome  $c_3$ .** The cytochrome  $c_3$  fraction from the G-75 column had a volume of 394 ml and a purity coefficient, defined as  $(A_{553}[\text{red.}] - A_{570}[\text{red.}]) / A_{280}[\text{ox.}]$ , of 3.03. The cytochrome was adsorbed on an Amberlite CG-50 type II column (4 by 15 cm) equilibrated with 10 mM Tris-hydrochloride buffer and eluted stepwise with 50 ml each of 50, 100, 200, 300, and 500 mM Tris-hydrochloride buffer. The cytochrome was eluted in a 110-ml fraction,

and its purity coefficient was 3.10. Finally, the cytochrome was filtered through a G-50 Sephadex column (5 by 200 cm) equilibrated with 10 mM Tris-hydrochloride buffer. The cytochrome-containing fraction had a volume of 360 ml, and the purity coefficient was 3.30, higher than any coefficient for cytochrome  $c_3$  found so far. After dialysis against 20 liters of distilled water, the cytochrome was lyophilized. The yield was equal to 550 mg of cytochrome  $c_3$ , judged to be pure by polyacrylamide gel electrophoresis.

(iv) Purification of the non-heme iron proteins. A settled volume of diethylaminoethyl (DEAE)-cellulose, equal to 300 ml of DEAE-cellulose, was added to the extract obtained after the silica gel treatment used to remove the cytochromes, and the mixture was stirred overnight. The DEAE-cellulose was then decanted and washed several times with 10 mM Tris-hydrochloride buffer. The adsorbed acidic proteins were eluted with 1 M Tris-hydrochloride buffer, giving a volume of 480 ml. This extract was dialyzed against 10 liters of distilled water and placed on a DEAE-cellulose column (4 by 30 cm) equilibrated with 10 mM Tris-hydrochloride. The column was washed with 200 ml of 150 mM Tris-hydrochloride, and the protein was eluted with 1 M Tris-hydrochloride in a volume of 120 ml. The volume of the extract was brought to 1,200 ml by addition of distilled water, and the proteins were adsorbed on a second DEAE-cellulose column (4 by 25 cm) and eluted with a linear gradient (2,000 ml) from 200 mM to 1 M Tris-hydrochloride. The first colored protein to be eluted was found to be rubredoxin, and two other bands of more acidic proteins had the typical absorption spectrum of ferredoxin. In contrast to all *Desulfovibrio* species we have studied so far, no flavodoxin band was found, and the ferredoxin content of the extract was clearly higher in the Norway strain than in the others.

(a) Purification of the rubredoxin. After the second DEAE-cellulose column, the rubredoxin was contained in a volume of 160 ml. The volume of the extract was brought to 350 ml with distilled water, and the rubredoxin was adsorbed on a DEAE-cellulose column (4 by 30 cm). The proteins were eluted with a linear gradient (1,200 ml) of 250 to 500 mM Tris-hydrochloride, and the rubredoxin was collected in a volume of 220 ml. The extract was directly applied to a silica gel column (2.5 by 30 cm) equilibrated with 350 mM Tris-hydrochloride. The rubredoxin migrated slowly in the column and was eluted in a volume of 180 ml. It was then adsorbed on a calcinated alumina column (2.5 by 10 cm) equilibrated with 350 mM Tris-

hydrochloride; the column was washed with 50 ml of the same buffer, and the rubredoxin was eluted with 10 mM Tris-hydrochloride in a volume of 30 ml. The protein was judged to be pure both from its spectrum ( $A_{280}/A_{490} = 2.35$ , identical to other rubredoxins from desulfovibrioness) and from polyacrylamide gel analysis; it was then dialyzed against 10 liters of distilled water and lyophilized, giving a yield of 45 mg. Some of its spectral characteristics are reported in Table 1.

(b) Purification of ferredoxin. The ferredoxin is acidic and was eluted with 500 mM Tris-hydrochloride (in a volume of 175 ml) from the second DEAE-cellulose column. The ferredoxin fraction was placed on a calcinated alumina column (5 by 25 cm), equilibrated with 500 mM Tris-hydrochloride, in order to remove a contaminant with a strong absorption at 260 nm. The ferredoxin was collected in the same buffer in a volume of 170 ml, and the volume was brought to 250 ml with distilled water. The ferredoxin was then adsorbed on a DEAE-cellulose column (4 by 25 cm) and eluted with a linear gradient (800 ml) from 350 to 550 mM Tris-hydrochloride. It was collected in a volume of 200 ml and placed on a calcinated alumina column (2 by 15 cm), equilibrated with 500 mM Tris-hydrochloride, in order to remove the last traces of the 260-nm contaminant, and was collected in a volume of 195 ml. The volume was brought to 240 ml with distilled water, and the ferredoxin was adsorbed on a DEAE-Sephadex column (2 by 25 cm) equilibrated with 400 mM Tris-hydrochloride. The protein was eluted with a linear gradient (300 ml) from 400 to 600 mM Tris-hydrochloride and collected in a volume of 74 ml. It was dialyzed against 2 liters of

TABLE 1. Molar extinction coefficients of rubredoxin, ferredoxin, and cytochrome  $c_3$  from *D. desulfuricans* Norway

Protein <sup>a</sup>	$\lambda$ /nm	$\epsilon$ /mol per cm
Rubredoxin (ox.)	490	6,900
	375.5	8,142
	279	15,870
Ferredoxin (ox.)	390	17,500
	305	20,588
Cytochrome $c_3$ (ox.)	531.5	50,132
	408.5	530,918
	351	118,737
Cytochrome $c_3$ (red.)	552	128,481
	523	662,46
	418.5	891,944

<sup>a</sup> ox., Oxidized; red., reduced.

distilled water, concentrated, and precipitated by dialysis against 500 ml of saturated ammonium sulfate solution. The precipitate was collected by centrifugation, washed in saturated ammonium sulfate, and finally resuspended in 10 ml of the same solution. The precipitate was dissolved by the addition of a few drops of distilled water, and a few crystals of ammonium sulfate were added to the solution. Crystals were formed in a few hours. This crystallization process was repeated twice. A small fraction of the crystallized ferredoxin was then dialyzed against distilled water, and the protein was judged to be pure from its spectrum (ratio  $A_{305}/A_{390} = 0.84$ , identical to ferredoxins from other desulfobacteriales) and by polyacrylamide gel analysis. The ferredoxin was usually kept in a crystalline form in ammonium sulfate solution and dialyzed against distilled water just prior to its utilization. The yield was 55 mg of pure ferredoxin.

The absorption spectrum of ferredoxin shows two peaks, one at 305 nm and the other at 390 nm, with a shoulder at 290 nm. The spectral properties are similar to those of the *D. gigas* ferredoxin (15). The molar extinction coefficient is 17,500 at 390 nm (see Table 1).

(v) Coupling activity of different electron carriers between hydrogenase and thiosulfate reductase. The coupling activities between hydrogenase and thiosulfate reductase of cytochrome  $c_3$  and ferredoxin from *D. desulfuricans* and *D. gigas* were tested using reductase preparations devoid of electron carriers from both microorganisms. The concentrations were chosen to give maximal activity. In addition, the activity of *D. gigas* cytochrome  $c_3$  (MW 26,000) in this reaction was compared with that obtained with the above-mentioned electron carriers. Cytochrome  $c_3$  from *D. desulfuricans* is an efficient carrier in this reaction with enzymatic preparation from the same organism, whereas *D. gigas* cytochrome  $c_3$  is inactive with the *D. desulfuricans* thiosulfate reductase and with *D. gigas* gives only a slight stimulation of thiosulfate reductase independent of the concentration, as already reported (11) (Fig. 1 and 2). The activity exhibited by cytochrome  $c_3$  from *D. desulfuricans* increases with increased cytochrome concentrations, and the maximum activity is reached with 24 nmol of cytochromes, using the same amount of reductase as in the experiment reported in Fig. 1. Both ferredoxins from *D. desulfuricans* and *Desulfovibrio gigas* are carriers in the coupling between hydrogenase and thiosulfate reductase from *D. gigas*; however, the two ferredoxins are less efficient with the enzymatic preparation from *D. desul-*

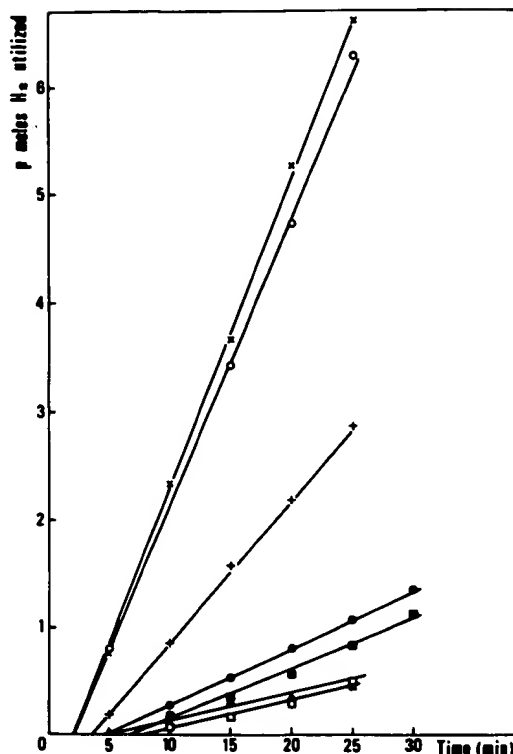


FIG. 1. Reduction of thiosulfate by  $H_2$  in the presence of various electron carriers with *D. desulfuricans* enzymatic preparation. The reaction mixture contained all the reactants indicated in Materials and Methods. Each flask contained enzymatic preparation (31 mg) and hydrogenase (400  $\mu$ g). Symbols: ( $\Delta$ ) control without added electron carrier; ( $\circ$ ) plus methyl viologen (100 nmol); plus *D. desulfuricans* cytochrome  $c_3$  (20 nmol); ( $\bullet$ ) plus *D. desulfuricans* ferredoxin (100 nmol); plus *D. gigas* cytochrome  $c_3$  (MW 13,000) (20 nmol); ( $\blacksquare$ ) plus *D. gigas* ferredoxin (100 nmol); ( $\times$ ) plus *D. gigas* cytochrome  $c_3$  (MW 26,000) (10 nmol).

*furicans*. Finally, as already reported (4), cytochrome  $c_3$  (MW 26,000) from *D. gigas* gives a strong stimulation with both enzymatic preparations of the same magnitude, on a molar basis, as that obtained with methyl viologen.

**Amino acid compositions.** The amino acid values were determined from the average of several acid hydrolysates. Corrections were made, when necessary, for the degradation or slow release of certain residues. The number of residues per mole of protein was determined by the mole ratio method.

(i) Ferredoxin. The amino acid composition and iron sulfide content of *D. desulfuricans* Norway ferredoxin are presented in Table 2. The protein contains 54 amino acids and is

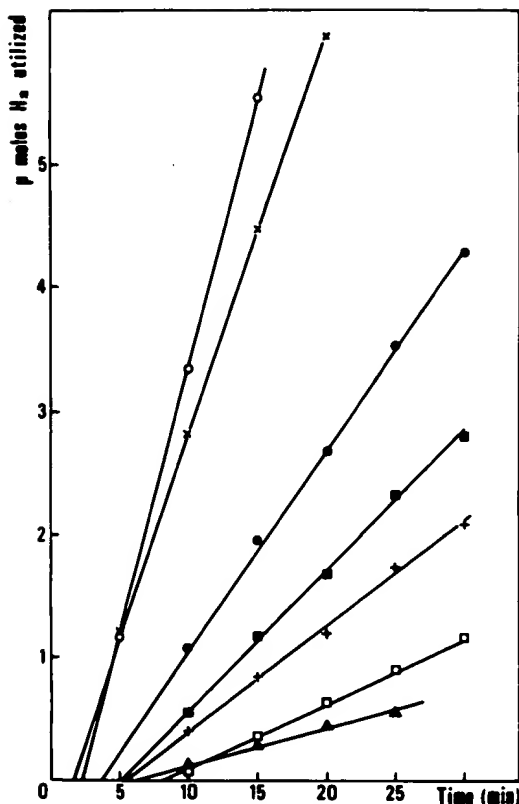


FIG. 2. Reduction of thiosulfate by  $H_2$  in the presence of various electron carriers with *D. gigas* enzymatic preparation. The reaction mixture contained all the reactants indicated in Materials and Methods. Each flask contained enzymatic preparation (22 mg of protein) and hydrogenase (400  $\mu$ g). Symbols: ( $\Delta$ ) control without added electron carrier; (O) plus methyl viologen (100 nmol); (+) plus *D. desulfuricans* cytochrome  $c_3$  (30 nmol); ( $\bullet$ ) plus *D. desulfuricans* ferredoxin (100 nmol); ( $\square$ ) plus *D. gigas* cytochrome  $c_3$  (MW 13,000) (30 nmol); ( $\blacksquare$ ) plus *D. gigas* ferredoxin (100 nmol); ( $\times$ ) plus cytochrome  $c_3$  (MW 26,000) (10 nmol).

devoid of histidine and tyrosine, like *D. gigas* ferredoxin (25). The minimum calculated molecular weight, including iron and sulfide, is 6,134. This value is in good agreement with the value estimated by polyacrylamide gel electrophoresis with sodium dodecyl sulfate, by reference with the migration of *D. gigas* ferredoxin (MW 6,056).

The analytical results show the presence of four iron atoms, four sulfide groups, and six cysteine residues.

The amino acid composition of the *Desulfovibrio* ferredoxins *D. gigas* and *D. desulfuricans* Berre S (29) show identical contents of half-cystine, labile sulfur, and iron. All the *Desul-*

*fovibrio* ferredoxins have a low content of basic amino acid residues and aromatic residues.

(ii) Rubredoxin. Hydrolysis of several samples of rubredoxin with 6 N hydrochloric acid under vacuum at 110°C for 20 h, followed by quantitative amino acid analysis, showed the amino acid content listed in Table 3. The amino acid compositions of the three *Desulfovibrio* rubredoxins so far reported are added for comparison. Acidic amino acids are preponderant, and all the rubredoxins are devoid of histidine and arginine residues, which is consistent with chromatographic properties on DEAE-cellulose. All the rubredoxins contain four cysteine residues, one non-heme iron, and no acid-labile sulfide. Calculation of molecular weight from the amino acid composition gave a result of 6,728, including one atom of iron per molecule.

(iii) Cytochrome  $c_3$ . Cytochrome  $c_3$ , first described by Postgate (22), is characteristic of the genus *Desulfovibrio*. It was then proposed that it contains two hemes per molecule, but from amino acid sequence analysis (1, 3; R. P. Ambler, Biochem. J. 109:47P-48P) it became clear that cytochrome  $c_3$  possesses four binding clusters for the hemes. Recent results of Yagi and Maruyama (28), Meyer et al. (18), and Dobson et al. (6) are consistent with the presence of four hemes per molecule. The amino acid composition of *D. desulfuricans* strain Norway cytochrome  $c_3$  is presented in Table 4 and compared with that obtained for cytochromes  $c_3$  from *D. vulgaris* strain Hildenborough, *D. gigas*, and *D. desulfuricans* strain E1 Agheila Z. The main characteristics of all cytochromes  $c_3$  are a high number of cysteine residues (8), of histidine residues (6-10) and of lysine residues.

After filtration on a G-50 Sephadex column, a molecular weight of 16,000 was determined. This figure is higher than the value of 13,000 determined for other  $c_3$ -type cytochromes and renders difficult a nomenclature of this type of hemoprotein based on molecular weight. The molecular weight based on the amino acid composition is 16,096. These values are consistent and give an average molecular weight for *D. desulfuricans* strain Norway cytochrome  $c_3$  of 16,000.

## DISCUSSION

The non-heme iron proteins of *D. desulfuricans*, Norway strain, do not differ in molecular weight, iron, and acid-labile sulfur content or in the number of cysteine residues from the other characterized *Desulfovibrio* ferredoxins and rubredoxins. Like the other bacterial rubredoxins, the rubredoxin is devoid of histidine and arginine residues and possesses four cys-



TABLE 2. Amino acid composition of *D. desulfuricans* Norway ferredoxin<sup>a</sup> and comparison with other *Desulfovibrio* sp.

Amino acid	Amino acid residues/molecule			
	<i>D. desulfuricans</i> (Norway) ferredoxin		Ferredoxin	
	From analysis	Nearest integer	<i>D. gigas</i> <sup>b</sup>	<i>D. desulfuricans</i> Berre S <sup>c</sup>
Lysine	1.8	2	1	2
Histidine	0	0	0	1
Ammonia				
Arginine	0	0	1	0
Tryptophan	ND	ND	0	0
Aspartic acid	4.8	5	11	10
Threonine	2.6	3	0	3
Serine	2.5	3	3	2
Glutamic acid	11	11	9	11
Proline	2.6	3	4	3
Glycine	2	2	1	6
Alanine	6.8	7	6	2
Cystine (half) <sup>d</sup>	6	6	6	6
Valine	3	3	5	5
Methionine <sup>d</sup>	1.9	2	2	0
Isoleucine	4.8	5	5	4
Leucine	1	1	1	2
Tyrosine	0	0	0	0
Phenylalanine	0.8	1	1	0
Total residues		54	56	57
Non-heme iron <sup>e</sup>		4	4	4
Acid-labile sulfide <sup>e</sup>		4	4	4

<sup>a</sup> Amino acid analyses were carried out using general methods with 20-h hydrolysis (6 N HCl) at 110°C under vacuum. Abbreviation: ND, Not determined.

<sup>b</sup> From Travis et al. (25).

<sup>c</sup> From Zubieta et al. (29).

<sup>d</sup> Cysteine was determined as cysteic acid after performate oxidation of the protein sample according to the method of Hirs (12).

<sup>e</sup> Atoms per molecule.

teine residues, one non-heme iron, and no acid-labile sulfide. The ferredoxin is devoid of histidine and tyrosine like *D. gigas* ferredoxin and has four iron atoms, four sulfide groups, and six cysteine residues. It is noteworthy that two ferredoxins were detected in the Norway strain. This second ferredoxin is more acidic and can be separated from the other on a DEAE-cellulose column. We have already noted that, by comparison with other *Desulfovibrio* species, ferredoxin is more abundant, and this seemed to be related to the absence of flavodoxin in the Norway strain. However, during the purification, it became clear that the second ferredoxin was quite unstable. As a result, only small amounts of this ferredoxin were recovered in a pure state, just enough for purity tests and amino acid determination. No reactivity experiments could be performed.

In earlier publications (2, 5), we have re-

ported the presence of three *c*-type cytochromes in *D. vulgaris* and *D. desulfuricans* (E1 Agh-eila Z), namely, *c*<sub>553</sub>, *c*<sub>3</sub> (MW 13,000), and *c*<sub>3</sub> (MW 26,000). The latter contain eight hemes per molecule; an analogous cytochrome was not detected in the Norway 4 strain.

The data on the coupling activity of *D. desulfuricans* (Norway 4) cytochrome *c*<sub>3</sub>, ferredoxin, and the homologous electron carriers of *D. gigas* between hydrogenase and thiosulfate reductase indicate that *D. desulfuricans* (Norway 4) cytochrome *c*<sub>3</sub> gives only a slight stimulation with the *D. gigas* reductase, as already reported (5). On the other hand, the ferredoxins of the two bacteria function as carriers in this reaction; however, these proteins are weakly active with the reductase preparation of *D. desulfuricans* (Norway 4). As described earlier (11), cytochrome *c*<sub>3</sub> (MW 26,000) from *D. gigas* appears to be the most efficient carrier in the

TABLE 3. Amino acid composition of *D. desulfuricans* Norway rubredoxin<sup>a</sup> and comparison with other *Desulfovibrio* rubredoxins

Amino acid	<i>D. desulfuricans</i> (Norway) rubredoxin		Rubredoxin		
	From analysis	Nearest integer	<i>D. gigas</i> <sup>b</sup>	<i>D. desulfuricans</i> <i>azotovorans</i> <sup>c</sup>	<i>D. vulgaris</i> <sup>d</sup>
Lysine	4.8	5	5	4	5
Histidine	0	0	0	0	0
Ammonia					
Arginine	0	0	0	0	0
Tryptophan	ND	ND	1	1	1
Aspartic acid	12.6	13	8	7	9
Threonine	3.5	4	2	2	2
Serine	0	0	4	2	4
Glutamic acid	5.2	5	5	8	5
Proline	5.1	5	5	6-7	7
Glycine	7.2	7	6	6	7
Alanine	5.0	5	5	6	6
Cystine (half) <sup>e</sup>	4	4	4	4	4
Valine	6.0	6	4	5	5
Methionine	0.82	1	1	1	1
Isoleucine	0	0	3	2	0
Leucine	0.94	1	2	0	1
Tyrosine	4.1	4	3	3	2
Phenylalanine	2.1	2	3	3	2
Total residues		63	61	60	61
Non-heme iron <sup>f</sup>		1	1	1	1
Acid-labile sulfide <sup>f</sup>		0	0	0	0

<sup>a</sup> Amino acid analyses were carried out using general methods with 20-h hydrolysis (6 N HCl) at 110°C under vacuum. ND, Not determined.

<sup>b</sup> From Laishley et al. (13).

<sup>c</sup> From Newman and Postgate (21).

<sup>d</sup> From Bruschi and Le Gall (4).

<sup>e</sup> Cysteine was determined as cysteic acid after performate oxidation of the protein sample according to the method of Hirs (12).

<sup>f</sup> Atoms per molecule.

coupling of hydrogenase with thiosulfate reductase. It should be emphasized that with our enzymatic preparation, devoid of electron carriers but containing thiosulfate and sulfite reductase activities, we used excess thiosulfate as substrate and measured only the reduction of thiosulfate, since sulfite is used only when all the thiosulfate has disappeared (11).

Wagner et al. (26) have reported the standard potential of thiosulfate reduction to sulfide and sulfite to be near -420 mV. This observation suggests that only a low-potential electron carrier could couple hydrogenase and thiosulfate reductase. The redox potential of *D. desulfuricans* cytochrome *c*<sub>3</sub> has been found to be in the vicinity of -500 mV (P. Bianco, personal communication), a high electronegative value as compared with the potential (-250 mV) reported for *D. gigas* cytochrome *c*<sub>3</sub> (MW 13,000) (16). Thus, in *D. desulfuricans* (Norway 4),

cytochrome *c*<sub>3</sub> is a good candidate for transferring electrons during thiosulfate reduction. However, in addition to the requirement for a low redox potential, the specificity of the electron carrier for hydrogenase and thiosulfate reductase is an important factor since ferredoxins from both bacteria, although extremely electronegative, give only a small stimulation at a concentration of 33.3 μM with the *D. desulfuricans* (Norway 4) reductase preparation. With this latter preparation the efficiency of *D. desulfuricans* (Norway 4) cytochrome *c*<sub>3</sub> in the coupling of hydrogenase with thiosulfate reductase is near that of *D. gigas* cytochrome *c*<sub>3</sub> (MW 26,000). In this case, a concentration of *D. desulfuricans* (Norway 4) cytochrome *c*<sub>3</sub> twice that of *D. gigas* cytochrome *c*<sub>3</sub> (MW 26,000) was used since, as already reported (5), the former cytochrome contains four hemes per molecule and the latter contains eight hemes. Then, in

TABLE 4. Amino acid composition of *D. desulfuricans* Norway cytochrome  $c_3$  (MW 13,000)<sup>a</sup> and comparison with other *Desulfovibrio* cytochromes

Amino acid	<i>D. desulfuricans</i> (Norway) cytochrome $c_3$		Cytochrome $c_3$ (MW 13,000)		
	From analysis	Nearest integer	<i>D. vulgaris</i> <sup>b</sup>	<i>D. gigas</i> <sup>c</sup>	<i>D. desulfuricans</i> El Agheila Z <sup>d</sup>
Lysine	15.99	16	20	17	15
Histidine	7.2	7-8	9	8	8
Ammonia					
Arginine	1.9	2	1	0	1
Tryptophan	0	0	0	1	1
Aspartic acid	15.1	15	12	18	8
Threonine	9.7	10	5	5	5
Serine	5.6	6	6	6	8
Glutamic acid	10.1	10	5	4	6
Proline	7.8	8	4	4	6
Glycine	9.7	10	9	11	8
Alanine	12.8	13	10	9	13
Cystine (half) <sup>e</sup>	8	8	8	8	8
Valine	6.0	6	8	8	5
Methionine	0.8	1	3	0	4
Isoleucine	3.6	4	0	4	2
Leucine	4.8	5	2	42	0
Tyrosine	1.2	2	3	2	1
Phenylalanine	3.8	4	2		3
Total residues		127	107	111	102

<sup>a</sup> Amino acid analyses were carried out using general methods with 20-h hydrolysis (6 N HCl) at 110°C under vacuum.

<sup>b</sup> From Ambler (Biochem. J. 109:47P-48P, 1968).

<sup>c</sup> From Ambler et al. (1).

<sup>d</sup> From Ambler et al. (3).

<sup>e</sup> Cysteine was determined as cysteic acid after performate oxidation of the protein sample according to the method of Hirs (12).

*D. desulfuricans* (Norway 4), cytochrome  $c_3$  replaces cytochrome  $c_3$  (MW 26,000) as an electron carrier in the electron transport system of thiosulfate reduction. These observations suggest that cytochromes  $c_3$ , although homologous proteins, have different physiological roles in the sulfate-reducing bacteria, since ferredoxin is not essential for coupling between hydrogenase and thiosulfate reductase in *D. desulfuricans* (Norway 4). In *D. gigas*, ferredoxin, flavodoxin, or cytochrome  $c_3$  (MW 26,000) is a carrier in the same reaction, when cytochrome  $c_3$  (MW 13,000) is almost inactive (11).

It can then be postulated that an important structural modification took place in *D. desulfuricans* (Norway 4) cytochrome  $c_3$ , allowing the disappearance of flavodoxin and the exclusion of ferredoxin from the electron transfer chain between hydrogenase and thiosulfate reductase so that the ferredoxin could be utilized in some other part of the complex electron transfer system typical of the sulfate-reducing bacteria. Comparison of the primary structure of other cytochromes  $c_3$  (3) shows that only 25%

of the total residues remain unchanged. Such a variability is not common in cytochromes of the  $c$  type and supports the hypothesis of changes in the function of cytochromes  $c_3$  in sulfate-reducing bacteria. The higher molecular weight of *D. desulfuricans* (Norway 4) cytochrome  $c_3$  is also in favor of this change. Both primary and tertiary structure determinations, which are now in progress (6, 9), will provide more precise information concerning the structure/function relationships in this class of hemoproteins.

#### ACKNOWLEDGMENTS

We are indebted to Jin-Po-Lee and A. Yi for the preparation of the extract of *D. desulfuricans* and M. Scandellari and R. Bourrelli (from the Laboratoire de Chimie Bactérienne) and the Fermentation Plant of the University of Georgia for growing the bacteria.

We gratefully acknowledge the technical assistance of Gisèle Bovier-Lapierre and Nicole Forget.

#### LITERATURE CITED

1. Ambler, R. P., M. Bruschi, and J. Le Gall. 1969. The structure of cytochrome  $c_3$  from *Desulfovibrio gigas* (N.C.I.B. 9332). FEBS Lett. 5:115-117.

2. Ambler, R. P., M. Bruschi, and J. Le Gall. 1971. Biochimie comparée des cytochromes des bactéries sulfato-réductrices, p. 25-34. In A. Perez-Niravete and D. Pelaez (ed.), Recent advances in microbiology, 10th International Congress for Microbiology, Mexico.
3. Ambler, R. P., M. Bruschi, and J. Le Gall. 1971. The amino acid sequence of cytochrome  $c_3$  from *Desulfovibrio desulfuricans* (strain El Agheila Z, N.C.I.B. 8380). FEBS Lett. 18:347-350.
4. Bruschi, M., and J. Le Gall. 1971. Purification et propriétés d'une rubrédoxine isolée à partir de *Desulfovibrio vulgaris* (souche N.C.I.B. 8303). Biochim. Biophys. Acta 263:279-282.
5. Bruschi, M., J. Le Gall, C. E. Hatchikian, and M. Dubourdieu. 1969. Cristallisation et propriétés d'un cytochrome intervenant dans la réduction du thiosulfate par *Desulfovibrio gigas*. Bull. Soc. Fr. Physiol. Veg. 15:381-390.
6. Dobson, C. M., N. J. Hoyle, C. F. Gerald, P. E. Wright, R. J. P. Williams, M. Bruschi, and J. Le Gall. 1974. An outline structure of cytochrome  $c_3$  and a consideration of its properties. Nature (London) 249:425-429.
7. Drucker, H., E. B. Trousil, L. L. Campbell, G. H. Barlow, and E. Margolish. 1970. Amino acid composition, heme content and molecular weight of cytochrome  $c_3$  of *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris*. Biochemistry 9:1515-1518.
8. Fogo, J. J., and M. Popowsky. 1949. Spectrophotometric determination of hydrogen sulfide, methylene blue method. Anal. Chem. 21:732-734.
9. Frey, M., R. Haser, M. Pierrot, M. Bruschi, and J. Le Gall. 1976. Preliminary crystallographic study of cytochrome  $c_3$  of *Desulfovibrio desulfuricans*. J. Mol. Biol. 104:741-743.
10. Harvey, A. E., J. A. Smart, Jr., and E. S. Amls. 1955. Simultaneous spectrophotometric determination of iron (II) and total iron with 1,10-phenanthroline. Anal. Chem. 27:26-35.
11. Hatchikian, E. C., J. Le Gall, M. Bruschi, and M. Dubourdieu. 1972. Regulation of the reduction of sulfite and thiosulfate by ferredoxin, flavodoxin and cytochrome  $c_3$  in extracts of the sulfate reducer *Desulfovibrio gigas*. Biochim. Biophys. Acta 258:701-708.
12. Hirs, C. H. W. 1967. Determination of cystine and cysteic acid, p. 59-62. In C. H. W. Hirs (ed.), Methods in enzymology, vol. 11. Academic Press Inc., New York.
13. Lashley, E. J., J. Travis, and H. D. Peck, Jr. 1969. Amino acid composition of ferredoxin and rubredoxin isolated from *Desulfovibrio gigas*. J. Bacteriol. 98:302-303.
14. Lee, J.-P., C.-S. Yi, J. Le Gall, and H. D. Peck, Jr. 1973. Isolation of a new pigment, desulforubidin, from *Desulfovibrio desulfuricans* (Norway strain) and its role in sulfite reduction. J. Bacteriol. 115:453-455.
15. Le Gall, J., and N. Dragoni. 1966. Dependence of sulfite reduction on a crystallized ferredoxin from *Desulfovibrio gigas*. Biochem. Biophys. Res. Commun. 23:145-149.
16. Le Gall, J., G. Mazza, and N. Dragoni. 1965. Le cytochrome  $c_3$  de *Desulfovibrio gigas*. Biochim. Biophys. Acta 56:385-387.
17. Lovenberg, W., B. B. Buchanan, and J. C. Rabinowitz. 1963. Studies on the chemical nature of clostridial ferredoxin. J. Biol. Chem. 238:3899-3913.
18. Meyer, T., R. G. Bartsch, and M. D. Kamen. 1971. A class of electron transfer heme-proteins found in both photosynthetic and sulfate-reducing bacteria. Biochim. Biophys. Acta 245:453-464.
19. Miller, J. D. A., and A. M. Saleh. 1964. A sulfite-reducing bacterium containing cytochrome  $c_3$  but lacking desulfoviridin. J. Gen. Microbiol. 37:419-423.
20. Moore, S., and W. H. Stein. 1963. Chromatographic determination of amino acids by the use of automatic recording equipment, p. 819-831. In S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 6. Academic Press Inc., New York.
21. Newman, D. J., and J. R. Postgate. 1968. Rubredoxin from a nitrogen-fixing variety of *Desulfovibrio desulfuricans*. Eur. J. Biochem. 7:45-50.
22. Postgate, J. R. 1956. Cytochrome  $c_3$  and desulfoviridine: pigments of the anaerobe *D. desulphuricans*. J. Gen. Microbiol. 14:545-572.
23. Postgate, J. R., and L. L. Campbell. 1966. Classification of *Desulfovibrio* species, the nonsporulating sulfate-reducing bacteria. Bacteriol. Rev. 30:732-738.
24. Shioi, Y., K. Takamiya, and M. Nishimura. 1972. Studies on energy and electron transfer systems in green photosynthetic bacterium *Chloropseudomonas ethylica* strain 2K. J. Biochem. 71:285-294.
25. Travis, J., D. J. Newman, J. Le Gall, and H. D. Peck, Jr. 1971. The amino acid sequence of ferredoxin from the sulfate reducing bacterium, *Desulfovibrio gigas*. Biochem. Biophys. Res. Commun. 45:452-458.
26. Wagner, G. C., R. J. Kassner, and M. D. Kamen. 1974. Redox potentials of certain vitamins K: implications for a role in sulfite reduction by obligately anaerobic bacteria. Proc. Natl. Acad. Sci. U.S.A. 71:253-256.
27. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.
28. Yagi, T., and K. Maruyama. 1971. Purification and properties of cytochrome  $c_3$  of *Desulfovibrio vulgaris*, Miyazaki. Biochim. Biophys. Acta 243:214-224.
29. Zubleta, J. A., R. Mason, and J. R. Postgate. 1973. A four iron ferredoxin from *Desulfovibrio desulfuricans*. Biochem. J. 133:851-854.

## Analysis of the Transcriptional Unit Encoding the Genes for Rubredoxin (*rub*) and a Putative Rubredoxin Oxidoreductase (*rbo*) in *Desulfovibrio vulgaris* Hildenborough

MICHAEL J. BRUMLIK AND GERRIT VOORDOUW\*

Division of Biochemistry, Department of Biological Sciences, The University of Calgary, Calgary, Alberta T2N 1N4, Canada

Received 28 March 1989/Accepted 6 June 1989

The nucleotide sequence of a 2.0-kilobase-pair *Eco*RI restriction fragment upstream from the gene (*rub*, 162 base pairs) encoding rubredoxin from *Desulfovibrio vulgaris* Hildenborough indicates that it is part of a larger transcriptional unit, containing an additional 378-base-pair open reading frame which terminates 16 nucleotides from the translational start of the *rub* gene and could encode a polypeptide of 14 kilodaltons (kDa). Northern (RNA) blotting of RNA isolated from both *D. vulgaris* Hildenborough and *Escherichia coli* TG2 transformed with plasmid pJK29, which contains both genes on a 1.1-kilobase-pair *Sa*I insert, confirms that the genes for this 14-kDa polypeptide and rubredoxin are present on a single transcript of 680 nucleotides. Strong evidence that the 14-kDa polypeptide is also a redox protein is provided by the fact that its NH<sub>2</sub> terminus is homologous to desulfuredoxin, which has been isolated from *D. gigas* as a small dimeric redox protein (36 amino acids per monomer), coordinating two iron atoms. Since rubredoxin is a potential redox partner for the 14-kDa protein, it has been tentatively named rubredoxin oxidoreductase, produced by the *rbo* gene. Southern blotting indicates that the *rbo-rub* operon is present in several species and strains of sulfate-reducing bacteria.

Rubredoxins are small, electron-carrying proteins (molecular mass, 6 kilodaltons [kDa]), which are found in the cytoplasm of several anaerobic bacteria, e.g., *Desulfovibrio gigas* (8), *D. vulgaris* Hildenborough (7), *D. desulfuricans* (15), *Thermodesulfobacterium commune* (24), *Clostridium pasteurianum* (36), *Peptostreptococcus elsdenii* (4), and *Peptococcus aerogenes* (3). The amino acid sequences of these rubredoxins have been determined (3, 4, 7, 8, 15, 36), and the three-dimensional structures of rubredoxins from *C. pasteurianum*, *D. vulgaris*, *D. gigas*, and *D. desulfuricans*, which has a very low molecular mass (only 5.2 kDa) have been solved (2, 13, 14, 32, 41-43). The redox center of rubredoxin consists of a single iron atom (redox potential, -50 to 0 mV), coordinated to four cysteinyl sulfurs. This redox potential is relatively high, since dissimilatory sulfate reduction by *Desulfovibrio* species requires electrons at -400 to -200 mV (17, 18, 23, 27), and it is not clear, therefore, which electron transfer reaction is catalyzed by rubredoxin. Several attempts have been made to define the redox partners of rubredoxin by biochemical experiments. By using the rubredoxin-mediated reduction of eucaryotic cytochrome *c* by NADH as an assay, an NADH-rubredoxin oxidoreductase was (partially) purified from *D. gigas* (16) and *C. acetobutylicum* (25). The *Clostridium* enzyme was found to have flavin adenine dinucleotide as the prosthetic group and to consist of a single subunit with a molecular mass of 41 kDa (25). The *D. gigas* enzyme shows specificity for rubredoxin from *D. gigas* ( $K_m = 6.2 \times 10^{-6}$  M) relative to rubredoxins from *D. vulgaris* ( $K_m = 5.3 \times 10^{-5}$  M) and *C. pasteurianum* ( $K_m = 1.0 \times 10^{-4}$  M), which show 71 and 63% sequence identity, respectively, with *D. gigas* rubredoxin. The subunit molecular mass and nature of the cofactor have not been reported for the *D. gigas* enzyme, and it has not been isolated from *D. vulgaris*.

A different approach to the identification of possible redox

partners for rubredoxin, which makes use of the fact that the gene encoding rubredoxin (*rub*) from *D. vulgaris* Hildenborough was recently cloned from a  $\lambda$  library of the *D. vulgaris* chromosome and its nucleotide sequence was determined (37), is taken here. No typical promoter consensus sequence (29) was found in the region immediately upstream from the *rub* gene, which instead appears to contain the 3' end of another reading frame, indicating that the *rub* gene may be part of a larger transcriptional unit. Since at least one gene encoding a redox protein interacting with rubredoxin could be present on this larger transcript, the region upstream from the *rub* gene is characterized in detail in the present paper.

### MATERIALS AND METHODS

**Strains, vectors, and media.** The bacterial strains, plasmids, and cloning vectors used in this work are described in Table 1. For the isolation of RNA, *D. vulgaris* Hildenborough was grown anaerobically in a medium made up from the following solutions, described by Pfennig et al. (26): solution 1 (1 liter, kept under 90% N<sub>2</sub>-10% CO<sub>2</sub>), solution 2 (1 ml), solution 3 (1 ml), solution 4 (30 ml), solution 5 (3 ml), 15% (wt/vol) of sodium lactate (10 ml), solution 8 (1 ml), solution 9 (0.1 ml), and Wolin vitamins (10 ml) (44). This medium allows a high growth rate of *D. vulgaris*, and RNA can successfully be isolated from cells grown for 16 h at 30°C at a 5% (vol/vol) inoculum. Chromosomal DNA, isolated from 15 different species and strains of sulfate-reducing bacteria (see Table 2) after growth on Postgate medium C (27), was a gift from Helen Kent, AFRC Unit of Nitrogen Fixation, University of Sussex, Brighton, England. DNA from *D. vulgaris* Miyazaki was a gift from T. Yagi, Department of Chemistry, Shizuoka University, Shizuoka, Japan, and DNA from *D. salexigens* NCIMB 8365 was donated by D. W. S. Westlake, Department of Microbiology, University of Alberta, Edmonton, Canada. *Escherichia coli* TG2 was grown in TY medium (22) containing 10 g of tryptone, 5

\* Corresponding author.

TABLE 1. Bacterial strains and DNA vectors used in this study

Strain or vector	Genotype, phenotype, comments, reference
<b>Strains</b>	
<i>D. vulgaris</i> subsp. <i>vulgaris</i> Hildenborough <sup>a</sup>	NCIMB 8303; isolated from clay soil near Hildenborough, U.K. (27)
<i>E. coli</i> TG2 <sup>b</sup>	$\Delta(lac-pro) supE thi hsdM hsdR recA F' (traD36 proAB^+ lacZ\Delta M15^+)$ ; from T. J. Gibson.
<b>Vectors</b>	
pUC8, pUC9	Amp <sup>r</sup> (35)
pJK15	This study (Fig. 1)
pJK29	This study (Fig. 1)
pRbo1	This study (Fig. 1)
pRub1	This study (Fig. 1)

<sup>a</sup> Abbreviated as *D. vulgaris* Hildenborough. A bacteriophage  $\lambda$  gene library has been constructed for this organism (37).

<sup>b</sup> Constructed from *E. coli* JM101 [ $\Delta(lac-pro) supE thi F' (traD36 proAB^+ lacZ\Delta M15^+)$ ] by T. J. Gibson and M. D. Biggin, Laboratory of Molecular Biology, Medical Research Council Centre, Cambridge, U.K.

g of yeast extract, and 5 g of NaCl per liter of water at pH 7.4.

**Biochemical reagents.** All enzymes were obtained from Pharmacia, Inc., with the exception of calf alkaline phosphatase, which was from Boehringer Mannheim Biochemicals. The radioisotopes [ $\alpha$ -<sup>35</sup>S]dATP (400 Ci/mmol; 10 mCi/ml), [ $\alpha$ -<sup>32</sup>P]dATP (3,000 Ci/mmol; 10 mCi/ml), and [ $\gamma$ -<sup>32</sup>P]ATP (3,000 Ci/mmol; 10 mCi/ml) were purchased from Amersham Corp. and were used for dideoxynucleotide sequencing, nick translation, and 3' and 5' end labeling, respectively. Ficoll 400 was purchased from Pharmacia, Inc. Polyvinylpyrrolidone (molecular weight 40,000), bovine serum albumin (fraction V), molecular biology grade sodium

dodecyl sulfate (SDS), bakers' yeast tRNA, salmon sperm DNA (sodium salt), and dextran sulfate (molecular weight 500,000) were purchased from Sigma Chemical Co. Low- and high-gelling-temperature (LGT and HGT) agarose were obtained from Bethesda Research Laboratories, Inc. Nitrocellulose and Hybond-N hybridization transfer membranes were obtained from Schleicher & Schuell, Inc., and Amersham, respectively. All other reagent grade chemicals were purchased from either Sigma or Fisher Scientific Co.

**DNA cloning.** Several recombinant bacteriophages carrying the *rub* gene were isolated from a  $\lambda$  library, as described previously (37). These clones cover 35 kilobase pairs (kb) of the *D. vulgaris* Hildenborough chromosome, and an *Eco*RI restriction map of a part of this region is shown in Fig. 1. A 2.0-kb *Eco*RI fragment and a 1.1-kb *Sal*I fragment (Fig. 1) were cloned into the *Eco*RI and *Sal*I sites of pUC8 (35), generating plasmids pJK15 and pJK29, respectively, which were purified as described elsewhere (40).

**Shotgun nucleotide sequencing.** The procedure followed for shotgun nucleotide sequencing was essentially that outlined by Bankier and Barrell (5). Plasmid pJK15 was sonicated, and the resulting fragments were end repaired and size fractionated by gel electrophoresis on 1% (wt/vol) LGT agarose. The 400- to 1,000-base-pair (bp) fraction was excised and isolated from the gel and ligated to the replicative form of M13mp8 (21), digested with *Sma*I and calf alkaline phosphatase (5). After being transfected into competent, CaCl<sub>2</sub>-treated *E. coli* TG2 cells, the ligation mixtures were spread onto TY plates with top agar, containing isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal) (5). Single-stranded DNA was isolated from white recombinant phage plaques by using a 1.5-ml miniprep procedure (5). The purified DNAs (2  $\mu$ l) were spotted on a nitrocellulose filter, which was subsequently baked at 80°C for 1 h under vacuum. The 2.0-kb *Eco*RI insert from pJK15 was isolated by LGT agarose gel electrophoresis, radiolabeled by nick translation

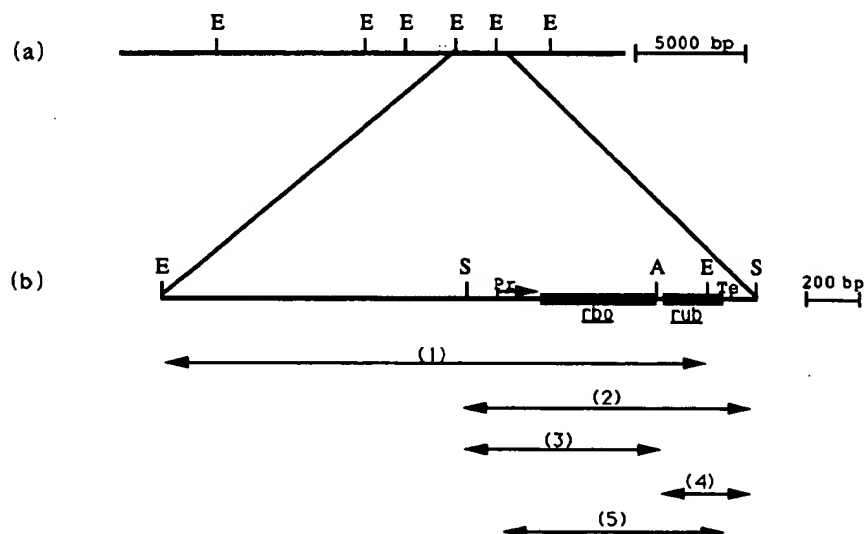


FIG. 1. Survey of DNA containing the *rub* gene of *D. vulgaris* Hildenborough. (a) Location of *Eco*RI (E) restriction sites as determined earlier (37). (b) Map of a 2,234-bp *Eco*RI-*Sal*I region of DNA for which the nucleic acid sequence was determined. The location of restriction sites for *Eco*RI, *Sal*I (S), and *Ava*I (A), the coding regions for the genes encoding rubredoxin (*rub*) and the putative rubredoxin oxidoreductase (*rbo*), and the location of promoter (Pr) and transcription terminator (Te) sequences, are indicated. The following plasmids (inserts) were constructed: 1, pJK15 (2.0 kb EE); 2, pJK29 (1.1 kb SS); 3, pRbo1 (746 bp AS) and 4, pRub1 (347 bp AS). The position of the nucleic acid sequence shown in Fig. 3 is indicated in region 5.

(20), and denatured by boiling. The filter was prehybridized in  $6\times$  SSC ( $1\times$  SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.2])– $10\times$  Denhardt solution (20)–0.5% (wt/vol) SDS for 15 min at 68°C. The 2.0-kb DNA probe was then added to the prehybridization solution, and hybridization was continued for 16 h at 68°C. After hybridization, the filter was washed in  $6\times$  SSC–0.5% (wt/vol) SDS at 68°C for 1 h. The filter was then dried and wrapped in Saran wrap, and positive clones were identified by autoradiography. These were then sequenced by the dideoxy-chain termination procedure developed by Sanger et al. (31), as detailed by Bankier and Barrell (5). The sequencing data were processed and analyzed by using the programs of Staden and McLachlan (33, 34).

**Construction of gene-specific probes.** The completed nucleotide sequence indicated the presence of a unique *Ava*I site in the 19-bp region separating the *rbo* and *rub* genes (Fig. 1; see also Fig. 3). Plasmid pJK29 was digested with *Ava*I, and the digested DNA was end repaired with the Klenow fragment of DNA polymerase I to generate blunt ends (20). Following inactivation of the polymerase at 68°C, pJK29 was further digested with *Sal*I, resulting in the excision of both the *rbo* and *rub* genes on 746- and 347-bp fragments of DNA, respectively; these were isolated by electrophoresis on 1.5% (wt/vol) LGT agarose. The purified fragments were ligated into pUC8, previously digested with *Sal*I and *Sma*I, to give plasmids pRbo1 and pRub1, respectively. The insert of pRbo1 was gel isolated following digestion with *Hind*III and *Eco*RI as a 767-bp *rbo* gene-specific probe, whereas the presence of an *Eco*RI site near the 3' end of the *rub* gene (Fig. 1; see also Fig. 3) allowed the isolation of a 162-bp *rub* gene-specific probe by digestion of pRub1 with *Eco*RI and gel electrophoresis.

**RNA isolation and Northern blotting.** RNA was isolated from cultures of *D. vulgaris* Hildenborough and *E. coli* TG2 by the hot-phenol extraction method (11) and stored as the ethanol precipitate at –20°C. Samples of RNA were collected by centrifugation, washed with 70% (vol/vol) ethanol, dried under vacuum, and redissolved in 20  $\mu$ l of loading buffer, prepared as described elsewhere (10). For Northern (RNA) blotting, RNA samples were electrophoresed on gels containing 1.0% (wt/vol) HGT agarose in  $1\times$  MOPS buffer ( $10\times$  MOPS buffer is 0.2 M 3-*N*-morpholinopropanesulfonic acid, 10 mM EDTA, and 50 mM sodium acetate [pH 7.0]) containing 2% (vol/vol) formaldehyde and then blotted onto a Hybond-N hybridization transfer membrane. Following transfer, the membrane was exposed to shortwave UV light to cross-link the RNA to the filter. Blots were prehybridized for 4 h at 42°C in a solution containing 0.75 M NaCl, 0.075 M sodium citrate (pH 7.0), 50% (vol/vol) formamide, 1% (wt/vol) SDS, 0.1% (wt/vol) Ficoll 400, 0.1% (wt/vol) polyvinylpyrrolidone, 0.1% (wt/vol) bovine serum albumin, and 0.16 mg of boiled bakers' yeast tRNA per ml. Following prehybridization, the blots were hybridized at 42°C for 16 h in the solution described above, also containing 10% (wt/vol) dextran sulfate as a hybridization enhancer, with a nick-translated probe derived from either plasmid pRbo1 or pRub1. Following hybridization, the blots were washed twice for 5 min at room temperature with 100 ml of 0.3 M NaCl–0.06 M Tris hydrochloride (pH 8.0)–0.002 M EDTA, then twice for 15 min at 60°C with 100 ml of the same solution with 0.5% (wt/vol) of SDS added, and, finally, twice for 15 min at room temperature with 0.003 M Tris base, after which the filters were dried and autoradiographed.

**S1 nuclease mapping.** A probe covering the region upstream from the *rbo* gene was prepared by digestion of

pJK29 with *Eco*RI and calf alkaline phosphatase and gel isolation of the 913-bp *Eco*RI fragment, containing the relevant *Sal*I–*Eco*RI fragment. The probe was 5' end labeled by adding T4 polynucleotide kinase (1  $\mu$ l; 10 U/ $\mu$ l) and [ $\gamma$ - $^{32}$ P]ATP (2.5  $\mu$ l; 10  $\mu$ Ci/ $\mu$ l) in the presence of 50 mM Tris hydrochloride (pH 7.6)–10 mM MgCl<sub>2</sub>–3 mM dithiothreitol–0.1 mM EDTA in a total volume of 10  $\mu$ l at room temperature for 1 h. The labeled probe was phenol extracted, ethanol precipitated, and dissolved in 20  $\mu$ l of 80% (vol/vol) formamide–20 mM PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid)] (pH 6.5)–400 mM NaCl (hybridization buffer). RNA (100  $\mu$ g) was dissolved in 40  $\mu$ l of hybridization buffer and incubated with the probe at 44°C for 12 h. Following the addition of 240  $\mu$ l of S1 nuclease buffer (300 mM NaCl, 18 mM sodium acetate [pH 4.6], 10 mM ZnSO<sub>4</sub>) and 2  $\mu$ l of S1 nuclease (384,000 U/ $\mu$ l), the hybridization solution was incubated at room temperature for 30 min. The reaction was terminated by the addition of 10  $\mu$ l of denatured bakers' yeast tRNA (1  $\mu$ g/ $\mu$ l), and then phenol extraction and ethanol precipitation of the DNA-RNA hybrids for 1 h at –70°C were carried out. The vacuum-dried samples were dissolved in 10  $\mu$ l of formamide dye mixture, prepared as described by Bankier and Barrell (5), and heated at 80°C for 5 min before being loaded on either a 4 or 6% (wt/vol) denaturing acrylamide gel (5), which was run at 39 W for 2 to 3 h. A partial *Hinf*I digest of plasmid pUC8, labeled by filling the ends with Klenow polymerase in the presence of dGTP, dCTP, dTTP, and [ $\alpha$ - $^{32}$ P]dATP, was used as a molecular size marker (1,613, 913, 517, 396, 140, 75, and 65 nucleotides).

**Southern blotting.** Samples of genomic DNA (3 to 10  $\mu$ g) from 17 different species and strains of sulfate-reducing bacteria were digested with *Eco*RI, electrophoresed on a 0.7% (wt/vol) HGT agarose gel as described elsewhere (40), and blotted on Hybond-N hybridization transfer membrane. The DNA was bound to the membrane by UV cross-linking as described above, and the blots were prehybridized for 1 h at 42°C with a solution (pH 7.4) containing 50% (vol/vol) formamide, 1 M NaCl, 0.1% (wt/vol) sodium PP<sub>i</sub>, 0.2% (wt/vol) polyvinylpyrrolidone, 0.2% (wt/vol) bovine serum albumin, 0.2% (wt/vol) Ficoll 400, 10% (wt/vol) dextran sulfate, and 0.175 mg of denatured salmon sperm DNA per ml. A nick-translated pRub1 probe (20) was then added, and hybridization was continued for 16 h. The Southern blots were washed similarly to the Northern blots, dried, and autoradiographed. The pRub1 probe was removed by washing with 0.5 M NaOH–1.5 M NaCl for 30 min and neutralizing for 30 min with 1 M Tris hydrochloride (pH 8.0)–1.5 M NaCl. Removal of the probe was confirmed by autoradiography, after which the blots were rehybridized with the nick-translated pRbo1 probe.

## RESULTS

**Nucleotide sequence upstream from *rub*.** The nucleotide sequence of the 2.0-kb *Eco*RI fragment, containing the region upstream from the *rub* gene, was determined by the random cloning and dideoxy sequencing procedure of Bankier and Barrell (5), and these data were combined with the sequence of the *rub* gene (37) to obtain a sequence of 2,234 nucleotides (nt) extending from an *Eco*RI to a *Sal*I site (Fig. 1b). An identification of possible coding regions with the aid of the codon probability method of Staden and McLachlan (34) in this 2.2-kb sequence is shown in Fig. 2. Apart from the *rub* gene, this analysis indicates the presence of two other plausible genes in frames a and b. The amino acid sequences derived from these possible genes were compared

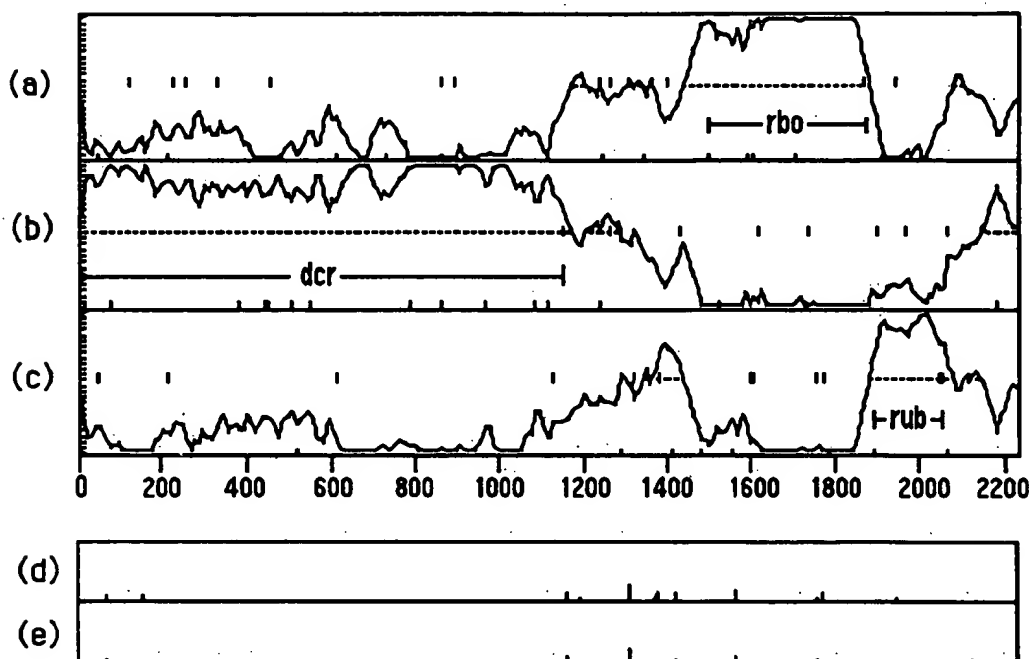


FIG. 2. Statistical analysis of the 2,234-bp region outlined in Fig. 1b. (a to c) The probability that a stretch of sequence (25 bases) is coding is calculated and plotted for each of the three reading frames a, b, and c (36). A codon usage table, which includes all codons of structural genes from *D. vulgaris* Hildenborough, was used as the standard in the calculation. Methionine (ATG) start codons are indicated on the base line, and stop codons are indicated at the half level of each frame. The coding regions for a putative rubredoxin oxidoreductase (*rbo*), a putative *D. vulgaris* chemoreceptor (*dcr*), and rubredoxin (*rub*) are indicated by the calculation as shown in frames a to c, respectively. (d and e) Search for *E. coli* promoters. Sequences with homology to the consensus  $-35$  (TTGACA) and  $-10$  (TATAAT) *E. coli* promoter sequence are indicated in frames d and e, respectively. The scale is in base pairs.

with sequences in the Bionet data base, which indicated unique and extensive homologies with, respectively, desulforedoxin of *D. gigas* (9) and the aspartate and serine chemoreceptors of *Salmonella typhimurium* (30) and *E. coli* (6), respectively. The amino acid sequence that can be derived from the sequenced portion of the *dcr* gene (nt 1 to 1158) lacks approximately 140 NH<sub>2</sub>-terminal amino acids, and further nucleic acid sequencing is required for the completion of the sequence of the putative *Desulfovibrio* chemoreceptor protein. The homology of the *dcr* gene product with these two known chemotactically active proteins will therefore not be further discussed here, allowing a focus on the gene immediately preceding the *rub* gene. In view of the homology with desulforedoxin, as discussed below, the upstream gene appears to also encode a redox protein and is tentatively referred to as rubredoxin oxidoreductase, the product of the *rbo* gene.

The nucleotide sequence of the *rub* gene and its upstream region (nt 1301 to 2140) is shown in Fig. 3. Each nucleotide was determined four times on average, at least once on each strand. The gene encoding rubredoxin (nt 1896 to 2057) and the putative *rbo* gene predicted by the codon probability calculations in Fig. 2a (nt 1499 to 1879) have been translated into amino acid sequences. Each gene is preceded by a plausible ribosome-binding site. A search for *E. coli* promoters (Fig. 2d and e) indicates the presence of a promoter consensus sequence ( $-35$ ,  $-10$ ) upstream from these two genes, and a potential promoter has been indicated in Fig. 3. A hairpin-loop-forming structure of nine G · C base pairs, which may serve as a transcription terminator, is present immediately downstream from the *rub* gene. Thus, an analysis of the nucleic acid sequence indicates that the two genes

may form an operon. Direct experimental evidence for the presence of both genes on a single transcript is presented below.

**Northern blotting and S1 nuclease mapping.** Northern blots of RNA isolated from both *D. vulgaris* and *E. coli* TG2(pJK29) indicate that a single transcript of approximately 680 nucleotides hybridizes with both the pRbo1 and the pRub1 probes (Fig. 4). The 1.1-kb insert of pJK29 is in the correct orientation for transcription of the *rbo* and *rub* genes from the *E. coli* *lac* promoter present on the pUC vector. However, a transcript originating from the *lac* promoter and containing both the *rbo* and *rub* genes would be approximately 1,100 nt long, and this size is not consistent with the data in Fig. 4C. The observation of similarly sized transcripts in *D. vulgaris* and *E. coli* indicates that the *D. vulgaris* promoter controlling the transcription of these two genes functions in *E. coli* and may thus resemble the *E. coli* consensus. The observed size of the transcript is sufficiently large to accommodate both the *rbo* and the *rub* genes. Assuming that the transcript terminates at position 2110, on the 3' side of the hairpin indicated in Fig. 3, the transcriptional start site is placed in the vicinity of position 1430, which is 70 nt upstream from the translational start of the *rbo* gene. The transcriptional start site was next defined more precisely by S1 nuclease mapping. Use of the 913-bp probe gives rise to a major protected fragment of  $600 \pm 50$  nt (average of three experiments), while minor protected fragments of variable, smaller sizes are also observed (Fig. 5). The 600-nt fragment ends at nt 2041 (Fig. 3), indicating a transcriptional start site at  $nt\ 1441 \pm 50$ . The presence of a promoter sequence with reasonable homology to the *E. coli* consensus at nt 1355 to 1380 (Fig. 3) suggests a potential start



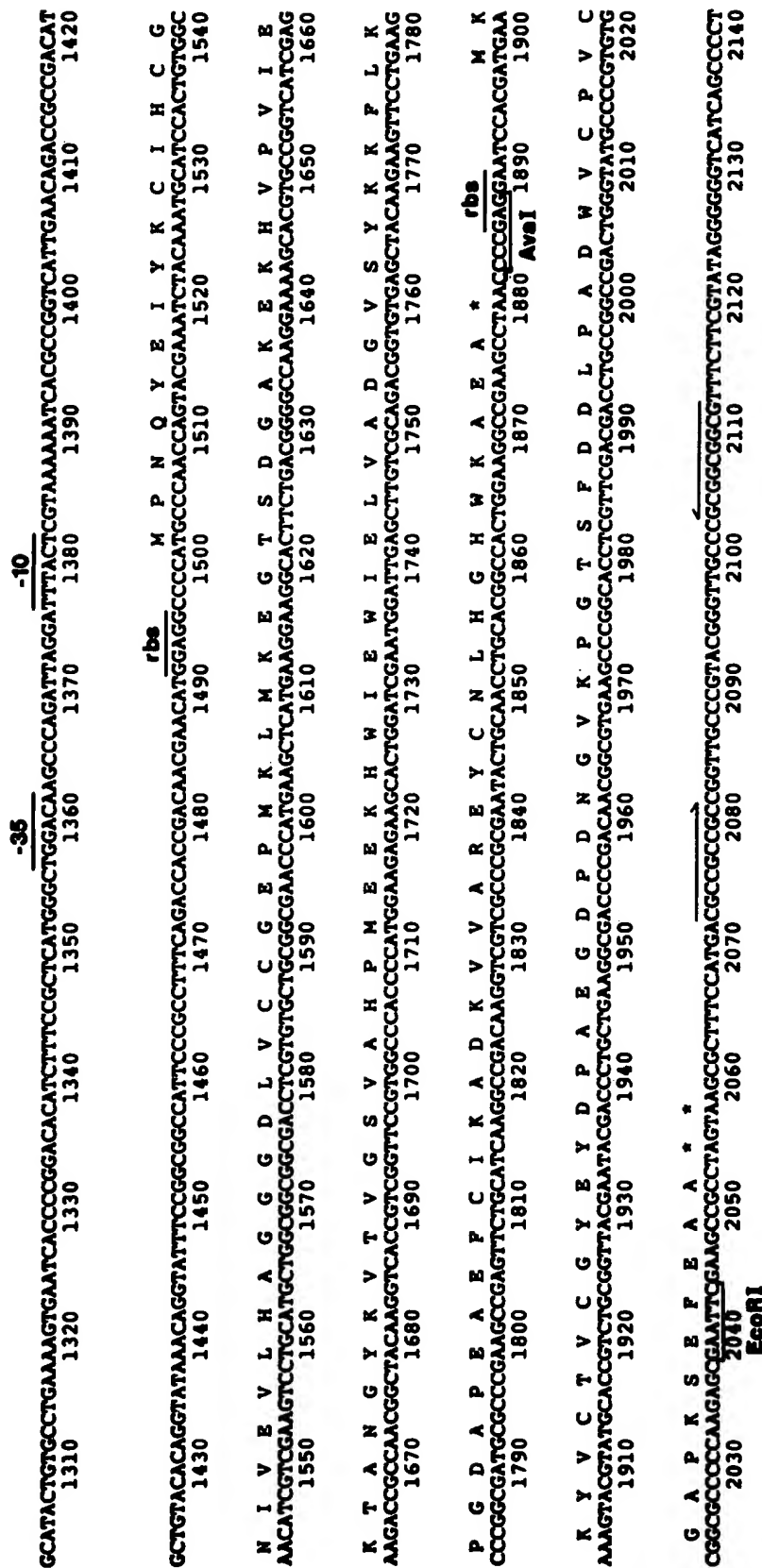


FIG. 3. Nucleotide sequence of the *rbo-rub* operon. The numbering of the sequence is the same as in Fig. 1 and 2. The coding regions of the *rbo* (nt 1499 to 1879) and *rub* (nt 1896 to 2057) genes have been translated with the single-letter amino acid code. Translation stop codons are designated by asterisks. The ribosome-binding sites (rbs) and a plausible promoter (−35, −10) are indicated. A G+C-rich stem-and-loop structure (---) which could serve as a transcription termination signal is shown.

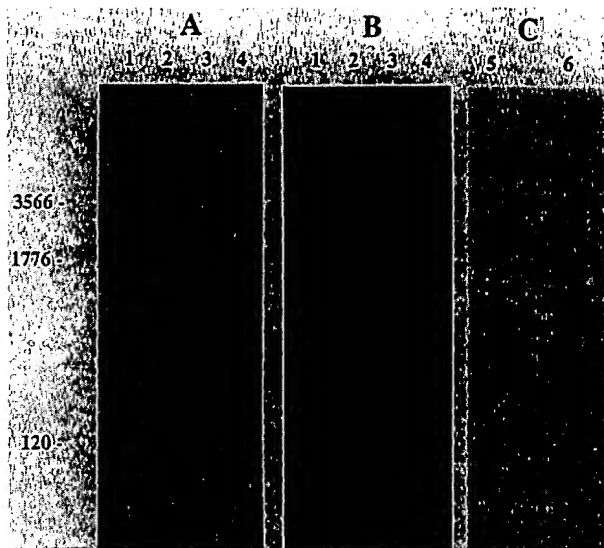


FIG. 4. Northern blotting of total RNA isolated from *D. vulgaris* Hildenborough and *E. coli* TG2(pJK29). (A) Increasing amounts of *D. vulgaris* RNA (25, 50, 75, and 100 µg) were loaded in lanes 1 to 4, respectively. The blot was hybridized to the pRb01 probe and autoradiographed for 5 days at  $-70^{\circ}\text{C}$ . The size markers, 25S rRNA (3,566 bases), 16S rRNA (1,776 bases), and 5S rRNA (120 bases), were visualized by fluorography of the blot following transfer of the ethidium bromide-stained gel. (B) The same blot as in panel A was incubated for 2 min in boiling distilled water to dissociate the pRb01 probe. Following autoradiography to confirm the dissociation of this probe, the blot was rehybridized to the pRub1 probe. Autoradiography was carried out for 7 days at  $-70^{\circ}\text{C}$ . (C) *D. vulgaris* RNA (25 µg) and RNA isolated from *E. coli* TG2 transformed with plasmid pJK29 (100 µg) were loaded in lanes 5 and 6, respectively. Following electrophoresis, the blot was incubated with the pRb01 probe. Autoradiography was performed for 14 days at  $-70^{\circ}\text{C}$ . Use of the pRub1 probe gave identical results (not shown).

site in the vicinity of nt 1390, which is within the range predicted by the S1 nuclease mapping results. The smaller (<600-nt) fragments do not map to the same position and are presumed to be artifacts arising from preferential S1 nuclease cleavage (12).

**Southern blotting.** Southern blots of chromosomal DNA digested with *Eco*RI from 17 different species and strains of sulfate-reducing bacteria, including *D. vulgaris* Hildenborough, were hybridized with the inserts from plasmids pRub1 and pRb01 as probes. The results obtained are shown in Fig. 6A and B, respectively, as well as in Table 2. These results are discussed in more detail below.

## DISCUSSION

**Homology of the *rbo* gene product with desulforedoxin.** Desulforedoxin is a small redox protein, which has been isolated from *D. gigas* but has so far not been found in other sulfate-reducing bacteria. Its polypeptide chain is only 36 amino acids long and has been determined by protein sequencing (9). As discussed by Le Gall et al. (18), the protein is isolated as a dimer (molecular mass, 7.9 kDa; 3.9 kDa per subunit) with two bound Fe atoms as redox centres and has rubredoxinlike spectroscopic properties. The product of the *rbo* gene (378 nt) is much larger than desulforedoxin (126 amino acids; molecular mass, 14 kDa). A comparison of the two amino acid sequences shows that the homology resides

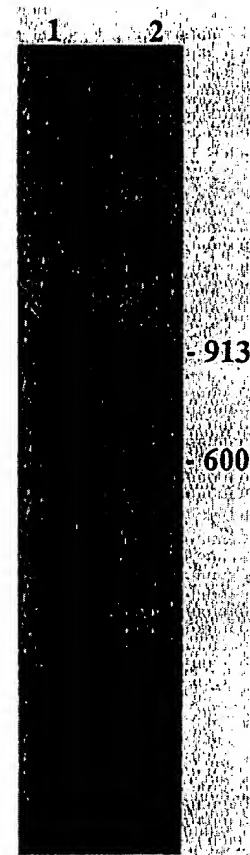


FIG. 5. S1 nuclease mapping of the transcriptional start site of the *rbo-rub* operon. Lanes: 1, 913-nt probe control; 2, *D. vulgaris* RNA (100 µg) hybridized with the 913-nt probe and treated with S1 nuclease; a major protected fragment of 600 nt is indicated. The size of this fragment was derived from the positions of pUC8  $\times$  *Hin*FI markers (not shown). Autoradiography was performed for 4 weeks at  $-70^{\circ}\text{C}$ .

entirely at the  $\text{NH}_2$  terminus (Fig. 7). Thus, of the first 36 amino acids of the *rbo* gene product, 19 are identical to desulforedoxin, whereas conservative amino acid changes are frequently found in the nonidentical positions. The four cysteine residues (C-9, C-12, C-28, and C-29), which have been proposed to coordinate to the iron (18), are among the conserved amino acid residues. Another interesting region of homology comprises the three glycine residues (G-22, G-23, G-24), which must represent an important flexible (e.g., loop) region of the molecule. The high degree of homology indicates that at its  $\text{NH}_2$  terminus, the *rbo* gene product must have a 4-kDa desulforedoxin domain, which has been fused to a larger polypeptide of 10 kDa. This situation is very reminiscent to that in [Fe] hydrogenase of *D. vulgaris* Hildenborough, which is known to have three iron-sulfur clusters as prosthetic groups coordinated by cysteine residues of the large (46-kDa) subunit. The  $\text{NH}_2$  terminus of this large subunit is homologous to bacterial 8Fe-8S ferredoxin (38), indicating that two electron-transferring 4Fe-4S clusters are likely to be present in this part of the molecule in a structure resembling that of ferredoxin (1), while a third unique hydrogen-binding cluster could be coordinated by some of the 10 cysteine residues found in the 40-kDa COOH-terminal part of the molecule. It was proposed, therefore, that the hydrogenase gene originated from a

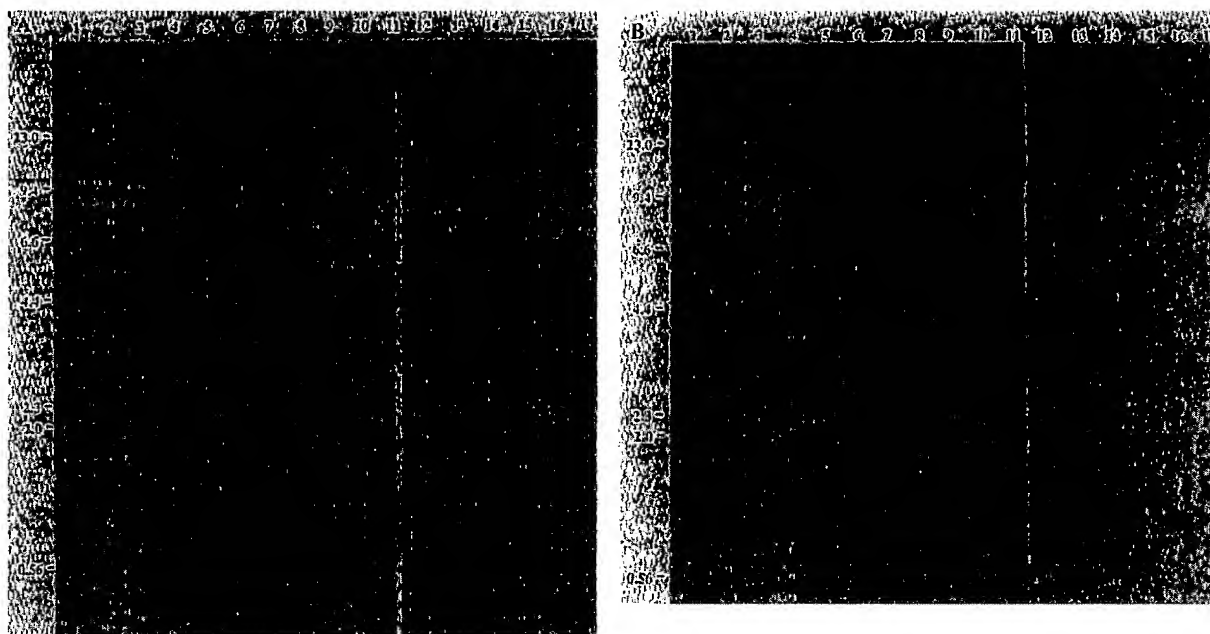


FIG. 6. Southern blotting of *Eco*RI-digested chromosomal DNA (5 to 10  $\mu$ g) from 17 species and strains of sulfate-reducing bacteria. Lanes: 1, *D. vulgaris* Hildenborough; 2, *D. vulgaris* Wandle; 3, *D. vulgaris* Brockhurst Hill; 4, *D. vulgaris* Groningen; 5, *D. vulgaris* Miyazaki; 6, *D. vulgaris* subsp. *oxamicus* Monticello; 7, *D. desulfuricans* Norway; 8, *D. desulfuricans* Teddington R; 9, *D. desulfuricans* El Agheila Z; 10, *D. desulfuricans* Berre Sol; 11, *D. desulfuricans* Canet 41; 12, *D. gigas*; 13, *D. salexigens* British Guiana; 14, *D. salexigens* California; 15, *D. salexigens*; 16, *D. africanus* Walvis Bay; 17, *D. africanus* Bhengazi. (A) The blots were hybridized with the pRub1 probe (containing part of the rubredoxin gene from *D. vulgaris* Hildenborough) and subjected to autoradiography for 8 days at  $-70^{\circ}\text{C}$ . The probe was then dissociated from the blots by washing in 0.5 M NaOH–1.5 M NaCl and neutralized by washing with 0.5 M Tris hydrochloride (pH 7.4)–1.5 M NaCl. Complete dissociation of the pRub1 probe was confirmed by autoradiography. (B) The blots were then hybridized with the pRbo1 probe (containing the gene of a putative rubredoxin oxidoreductase from *D. vulgaris* Hildenborough) and subjected to autoradiography for 2.5 days at  $-70^{\circ}\text{C}$ . The positions of molecular size markers (bacteriophage  $\lambda$  DNA digested with *Hind*III) are indicated in kilobases.

TABLE 2. Hybridization of *Eco*RI-digested chromosomal DNA from 17 different sulfate-reducing bacteria with an *rub*-specific and an *rbo*-specific probe from *D. vulgaris* Hildenborough

Species	Strain	NCIMB no.	Size of hybridizing fragment (kb) for:	
			<i>rub</i>	<i>rbo</i>
<i>D. vulgaris</i> subsp. <i>vulgaris</i>	Hildenborough	8303	2.0	2.0
	Wandle	8306	2.0	2.0
	Brockhurst Hill	8306	2.0	2.0
	Groningen	11779	15.4	15.4
	Miyazaki		0.5	3.9
<i>D. vulgaris</i> subsp. <i>oxamicus</i>	Monticello 2	9442	0.5	1.8
<i>D. desulfuricans</i> subsp. <i>desulfuricans</i>	Norway 4	8310		
	Teddington R <sup>a</sup>	8312		7.8
	El Agheila Z	8318		
	Berre Sol	8388	0.5	2.8
	Canet 41	8393	0.5	
<i>D. gigas</i>		9332	1.2	
<i>D. salexigens</i>	British Guiana	8403		
	California 43:63	8364		
		8365		
<i>D. africanus</i>	Walvis Bay	8397	7.1	
	Bhengazi	8401	0.4	3.0

<sup>a</sup> Reclassified as *D. vulgaris* (28).

fusion of genes encoding an electron-transferring ferredoxin and a larger hydrogen-binding polypeptide (38). In the present case, the desulforedoxin domain coincides precisely with the 36 NH<sub>2</sub>-terminal amino acids.

Another example of a redox protein that could have arisen by gene fusion is rubrerythrin, which was recently isolated (19) and shown to contain both rubredoxinlike and hemerythrinlike redox centres. The putative *rbo* gene product is distinct from rubrerythrin in its molecular mass (14 versus 22 kDa) and amino acid composition. Also, preliminary protein sequence data on rubrerythrin (D. M. Kurtz, Jr., and J. Le Gall, personal communication) indicate that this protein does not resemble the *rbo* gene product.

Two possible modes of coordination of the two iron atoms by the eight cysteines of a desulforedoxin dimer have been considered by Le Gall et al. (18): the four cysteines of a single subunit could coordinate to the same iron, in which case dimer formation is achieved by noncovalent interactions of the two subunits, or cysteines from both subunits (e.g., C-9, C-12, and C-28 from one and C-29 from the other) could contribute to the coordination of the two irons, causing a partially covalent connection of the two subunits via the two Fe redox centres. In view of its homology with desulforedoxin, the functional form of the *rbo* gene product is also proposed to be a dimer. Only two additional cysteine residues (C-103 and C-115) are present in the remainder of the sequence of the *rbo* gene product (Fig. 7), which is insufficient for the coordination of a third Fe-containing redox centre (e.g., the coordination of a single Fe, a single

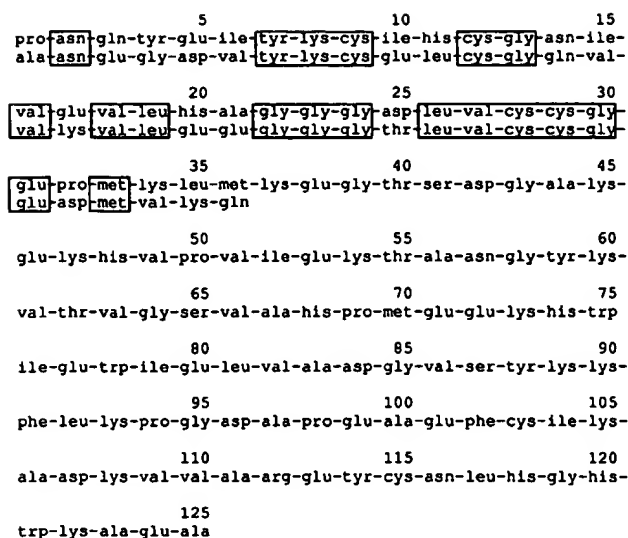


FIG. 7. Comparison of the amino acid sequence of the *rbo* gene product (Fig. 3) with that of desulfiredoxin (9). Regions of sequence identity are highlighted by boxes.

2Fe-2S cluster, or a single 4Fe-4S cluster requires four cysteines). However, a third redox centre could be present in the 14-kDa *rbo* gene product, if one assumes that the two subunits can donate both cysteine ligands to the same coordination site. The *rbo* gene product appears quite different from the NADH-rubredoxin oxidoreductase purified from *C. acetobutylicum* which was discussed in the Introduction. The subunit molecular mass is different (41 kDa for the *Clostridium* enzyme), and the search of the Bionet data base did not reveal homologies of the *rbo* gene product with flavin-binding proteins.

In analogy with the [Fe] hydrogenase gene, one could suggest that the *rbo* gene has arisen by fusion of the gene for desulfiredoxin with that encoding a 10-kDa redox protein containing a single redox centre. The observation that this gene forms a single operon with the gene encoding rubredoxin could mean that the two proteins are redox partners, but definitive proof for this proposal must await the purification and characterization of this novel redox protein.

**Distribution of the *rbo* gene in sulfate-reducing bacteria.** The *rub* and *rbo* genes are present on a similarly sized *EcoRI* fragment (Fig. 6) and may thus form an operon as in Fig. 3 in *D. vulgaris* Wandle, Brockhurst Hill, and Groningen. This is no surprise for the first two strains, which are practically identical to the Hildenborough strain (39). However, the Groningen strain is quite distinct from Hildenborough and was shown to lack a gene for [Fe] hydrogenase and to have only a low degree of homology in its gene for cytochrome *c*<sub>3</sub> (39). Four species of sulfate-reducing bacteria have homologous *rub* and *rbo* genes on different restriction fragments (Table 2; Fig. 6). This could indicate a separation of the two genes in these species or the presence of an additional *EcoRI* site in their *rbo-rub* operon. Finally, a homologous *rub* gene in the absence of a detectable, homologous *rbo* gene is found in three, whereas the converse is found in a single one of the species of sulfate-reducing bacteria examined. *D. gigas*, which has a rubredoxin with 71% sequence identity (37 identical residues in a total of 52) with the protein from *D. vulgaris* Hildenborough (36), the pRub1 probe appears sufficiently homologous for the detection of the *rub* gene on a 1.2-kb *EcoRI* fragment (Fig. 6A, lane 12; Table 2). However,

the 53% identity of *D. gigas* desulfiredoxin with the N terminus of the *D. vulgaris* *rbo* gene product (Fig. 7; 19 identical residues in a total of 36) appears insufficient for the detection of the desulfiredoxin gene in *D. gigas* (Fig. 6B, lane 12). The data in Fig. 6 should therefore not be interpreted in terms of the presence or absence of a gene encoding a desulfiredoxinlike polypeptide in species that fail to hybridize with the pRub1 probe. They indicate that the *rbo* gene is present in nine other species of sulfate-reducing bacteria and is spatially linked to the *rub* gene in at least four species. The *rbo* gene product could thus function as a redox protein in some but not all species of sulfate-reducing bacteria.

#### ACKNOWLEDGMENTS

M.J.B. acknowledges the support of Graduate Teaching and Graduate Research Assistantships from the University of Calgary. This work was supported by an establishment grant from the Alberta Heritage Foundation for Medical Research and an operating grant from the National Science and Engineering Research Council of Canada to G.V.

#### LITERATURE CITED

- Adman, E. T., L. C. Sieker, and L. H. Jensen. 1973. The structure of a bacterial ferredoxin. *J. Biol. Chem.* 248:3987-3996.
- Adman, E. T., L. C. Sieker, L. H. Jensen, M. Bruschi, and J. Le Gall. 1977. A structural model of rubredoxin from *Desulfovibrio vulgaris* at 2 Å resolution. *J. Mol. Biol.* 112:113-120.
- Bachmayer, H., A. M. Benson, K. T. Yasunobu, W. T. Garrard, and H. R. Whiteley. 1968. Non-heme iron proteins. IV. Structural studies of *Micrococcus aerogenes* (*Peptococcus aerogenes*) rubredoxin. *Biochemistry* 7:968-996.
- Bachmayer, H., J. L. Peel, K. T. Yasunobu, and S. G. Mayhew. 1968. Non-heme iron proteins. V. The amino acid sequence of rubredoxin from *Peptostreptococcus elsdenii*. *J. Biol. Chem.* 243:1024-1032.
- Bankier, A. T., and B. G. Barrell. 1983. Shotgun DNA sequencing, p. 1-34. In R. A. Flavell (ed.), *Techniques in the life sciences*, B5. Nucleic acid biochemistry. Elsevier Scientific Publishers Ireland Ltd., Shannon, Ireland.
- Boyd, A., K. Kendall, and M. Simon. 1983. Structure of the serine chemoreceptor in *Escherichia coli*. *Nature (London)* 301:623-626.
- Bruschi, M. 1976. Non-heme iron proteins: the amino acid sequence of rubredoxin from *Desulfovibrio vulgaris*. *Biochim. Biophys. Acta* 434:4-17.
- Bruschi, M. 1976. The amino acid sequence of rubredoxin from the sulfate-reducing bacterium *Desulfovibrio gigas*. *Biochem. Biophys. Res. Commun.* 70:615-621.
- Bruschi, M., I. Moura, J. Le Gall, A. V. Xavier, and L. C. Sieker. 1979. The amino acid sequence of desulfiredoxin, a new type of non heme iron protein from *Desulfovibrio gigas*. *Biochem. Biophys. Res. Commun.* 90:596-605.
- Davis, L. G., J. F. Battey, and M. P. Dibner. 1986. Basic methods in molecular biology, p. 143-146. Elsevier Science Publishing Co. Ltd., Amsterdam.
- Ebina, Y., and A. Nakazawa. 1983. Cyclic AMP-dependent initiation and p-dependent termination of colicin E1 gene transcription. *J. Biol. Chem.* 258:7072-7078.
- Favaloro, J., R. Treisman, and R. Kamen. 1980. Transcription maps of polyoma virus-specific RNA: analysis by 2-dimensional nuclease S<sub>1</sub> gel mapping. *Methods Enzymol.* 65:718-749.
- Frey, M., L. Sieker, F. Payan, R. Haser, M. Bruschi, G. Pepe, and J. Le Gall. 1987. Rubredoxin from *Desulfovibrio gigas*. A molecular model of the oxidized form of 1.4 Å resolution. *J. Mol. Biol.* 197:525-541.
- Herriott, J. R., L. C. Sieker, L. H. Jensen, and W. Lovenberg. 1970. Structure of rubredoxin: an X-ray study to 2.5 Å resolution. *J. Mol. Biol.* 50:391-406.

15. Hormel, S., K. A. Walsh, B. C. Prickril, K. Titani, J. Le Gall, and L. C. Sieker. 1986. Amino acid sequence of rubredoxin from *Desulfovibrio desulfuricans* strain 27774. *FEBS Lett.* 201:147-150.
16. Le Gall, J. 1968. Purification partielle et étude de la NAD: rubrédoxine oxydo-réductase de *D. gigas*. *Ann. Inst. Pasteur (Paris)* 114:109-115.
17. Le Gall, J., D. V. DerVartanian, and Harry D. Peck, Jr. 1979. Flavoproteins, iron proteins, and hemoproteins as electron-transfer components of the sulfate-reducing bacteria. *Curr. Top. Bioenerg.* 9:237-265.
18. Le Gall, J., J. J. G. Moura, H. D. Peck, Jr., and A. V. Xavier. 1982. Hydrogenase and other iron-sulfur proteins from sulfate-reducing and methane forming bacteria, p. 177-248. In T. G. Spiro (ed.), *Iron-sulfur proteins*. John Wiley & Sons, Inc., New York.
19. Le Gall, J., B. C. Prickril, I. Moura, A. V. Xavier, J. J. G. Moura, and B.-H. Huynh. 1988. Isolation and characterization of rubrerythrin, a non-heme iron protein from *Desulfovibrio vulgaris* that contains rubredoxin centers and a hemerythrin-like binuclear iron cluster. *Biochemistry* 27:1636-1642.
20. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. Messing, J., and J. Viera. 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. *Gene* 19:269-276.
22. Miller, J. H. 1977. Experiments in molecular genetics, 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. Odom, J. M., and H. D. Peck, Jr. 1984. Hydrogenase, electron transfer proteins, and energy coupling in the sulfate-reducing bacteria *Desulfovibrio*. *Annu. Rev. Microbiol.* 38:551-592.
24. Papavassilia, P., and E. C. Hatchikian. 1985. Isolation and characterization of a rubredoxin and a two-(4Fe-4S) ferredoxin from *Thermodesulfobacterium commune*. *Biochim. Biophys. Acta* 810:1-11.
25. Petitdemange, H., R. Marczak, H. Blusson, and R. Gay. 1979. Isolation and properties of reduced nicotinamide adenine dinucleotide-rubredoxin oxidoreductase of *Clostridium acetobutylicum*. *Biochem. Biophys. Res. Commun.* 91:1258-1265.
26. Pfennig, N., F. Widdel, and H. T. Trüper. 1981. Dissimilatory sulphate-reducing bacteria, p. 931-932. In M. P. Starr (ed.), *The prokaryotes*, vol. 1, Springer-Verlag, New York.
27. Postgate, J. R. 1984. The sulphate-reducing bacteria, 2nd ed., p. 1-82. Cambridge University Press, Cambridge.
28. Postgate, J. R., H. M. Kent, and R. L. Robson. 1986. DNA from diazotrophic *Desulfovibrio* strains is homologous to *Klebsiella pneumoniae* structural *nif* DNA and can be chromosomal or plasmid-borne. *FEMS Microbiol. Lett.* 33:159-163.
29. Rosenberg, M., and D. Court. 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. *Annu. Rev. Genet.* 13:319-353.
30. Russo, A. F., and D. E. Koshland, Jr. 1983. Separation of signal transduction and adaption functions of the aspartate receptor in bacterial sensing. *Science* 220:1016-1020.
31. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463-5467.
32. Sieker, L. C., R. E. Stenkamp, L. H. Jensen, B. Prickril, and J. Le Gall. 1986. Structure of rubredoxin from the bacterium *Desulfovibrio desulfuricans*. *FEBS Lett.* 208:73-76.
33. Staden, R. 1984. Graphical methods to determine the function of nucleic acid sequences. *Nucleic Acids Res.* 12:521-538.
34. Staden, R., and A. D. McLachlan. 1982. Codon preference and its use in identifying protein coding regions in long DNA sequences. *Nucleic Acids Res.* 10:141-156.
35. Viera, J., and J. Messing. 1982. The pUC plasmid, an M13mp7 derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene* 19:259-268.
36. Vogel, H., M. Bruschi, and J. Le Gall. 1977. Phylogenetic studies of two rubredoxins from sulfate reducing bacteria. *J. Mol. Evol.* 9:111-119.
37. Voordouw, G. 1988. Cloning of genes encoding redox proteins of known amino acid sequence from a library of the *Desulfovibrio vulgaris* (Hildenborough) genome. *Gene* 69:75-83.
38. Voordouw, G., and S. Brenner. 1985. Nucleotide sequence of the gene encoding the hydrogenase from *Desulfovibrio vulgaris* (Hildenborough). *Eur. J. Biochem.* 148:515-520.
39. Voordouw, G., H. M. Kent, and J. R. Postgate. 1987. Identification of the genes for hydrogenase and cytochrome  $c_3$  in *Desulfovibrio*. *Can. J. Microbiol.* 33:1006-1010.
40. Voordouw, G., J. E. Walker, and S. Brenner. 1985. Cloning of the gene encoding the hydrogenase from *Desulfovibrio vulgaris* (Hildenborough) and the determination of the  $\text{NH}_2$ -terminal sequence. *Eur. J. Biochem.* 148:509-514.
41. Watenpaugh, K. D., T. N. Margulis, L. C. Sieker, and L. H. Jensen. 1978. Water structure in a protein crystal: rubredoxin at 1.2 Å resolution. *J. Mol. Biol.* 122:175-190.
42. Watenpaugh, K. D., L. C. Sieker, and L. H. Jensen. 1979. The structure of rubredoxin at 1.2 Å resolution. *J. Mol. Biol.* 131:509-522.
43. Watenpaugh, K. D., L. C. Sieker, and L. H. Jensen. 1980. Crystallographic refinement of rubredoxin at 1.2 Å resolution. *J. Mol. Biol.* 138:615-633.
44. Wolin, E. A., M. J. Wolin, and R. F. Wolfe. 1963. Formation of methane by bacterial extracts. *J. Biol. Chem.* 238:2882-2886.

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**